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Volume 44
1947

PUBLISHERS
AMERICAN MEDICAL ASSOCIATION
CHICAGO, ILL.

Medical Lib.
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ARCHIVES OF PATHOLOGY

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STUDIES ON THE PATHOGENESIS OF RHEUMATIC FEVER

I. Experimental Production of Autoantibodies to Heart, Skeletal Muscle and Connective Tissue

PHILIP A. CAVELTI, M.D.
SAN FRANCISCO

IN 1934 Burky [1] showed that rabbits receiving injections of toxic filtrates of Staphylococcus aureus which had been grown on broth prepared from lens substance became sensitized to plain lens substance, as evidenced by cutaneous reactions. He was able to effect similar autosensitization to rabbit muscle extract by using as an immunizing agent a toxin prepared by growing the organisms on a medium containing rabbit muscle. Staphylococcus toxin, therefore, has the ability to confer antigenicity on substances of tissues of animals, with consequent formation of isoantibodies to these tissue substances within animals of the same species.

Using kidney in connection with staphylococcus toxin, Schwentker and Comploier [2] obtained complement-fixing antibodies to plain rabbit kidney. Streptococcus toxin was found by them to be only slightly effective in conferring antigenicity on rabbit kidney.

Precipitins to extracts of rabbit skin were produced by Hecht, Sulzberger and Weil [3] in rabbits by immunizing these animals with a combination of rabbit skin emulsion and staphylococcus toxin.

In our previous experiments [4] we have shown that autoantibodies to kidney can be produced both in rabbits and in rats by injecting renal material in combination with streptococcic substances, especially whole killed group A beta hemolytic streptococci. The autoantibodies were demonstrable in vitro (for rabbits, often in high titers) by means of the collodion particle technic. Further, in rats, as a result of the in vivo reaction of the autoantibodies with the kidney in situ, acute and chronic

This investigation was aided by grants from the Commonwealth Fund.

From the George Williams Hooper Foundation for Medical Research and the Division of Medicine of the University of California Medical School.

1. Burky, E. L.: J. Allergy **5**:466, 1934.
2. Schwentker, F. F., and Comploier, F. C.: J. Exper. Med. **70**:223, 1939.
3. Hecht, R.; Sulzberger, M. B., and Weil, H.: J. Exper. Med. **78**:59, 1943.
4. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **39**:148, 1945; **40**:158 and 163, 1945.

glomerulonephritis was produced. These results lend support to the concept that human glomerulonephritis also may be due to specific autoantibodies to kidney formed as a response to an antigen arising from a combination of streptococcic substance with renal material during the streptococcic infection which almost invariably precedes human glomerulonephritis by at least a few days, usually one to three weeks.

In the experiments to be reported here, the question was investigated whether streptococci, in an analogous manner, are able to render substances from certain other tissues antigenic, thus leading to the production of autoantibodies to these tissue substances. It was further studied whether any such autoantibodies formed are capable of damaging the corresponding tissues by their reaction with the antigen in situ. From a clinical point of view, rheumatic fever could conceivably be due to such mechanisms, as suggested by the relation of this disease to a preceding streptococcic infection. As these experiments were carried out with a view to the genesis of rheumatic fever, the tissues chiefly employed in this study were heart, connective tissue and muscle, the structures which are predominantly affected by the rheumatic lesions.

MATERIALS AND METHODS

Preparation of Tissue Materials.—For most of the experiments which were done on rats, tissues were employed which had previously been washed free of blood by perfusion of the whole animal with isotonic solution of sodium chloride under sterile conditions. With the rat under ether anesthesia, a cannula was introduced into the left ventricle immediately after the chest was opened, and, with a clip of the scissors, an opening was made in the right auricle. Two minutes usually were sufficient for perfusion of the heart, but when other tissues were to be used, perfusion was continued for about five to ten minutes.

Heart and kidneys were removed aseptically. To obtain skeletal muscle and connective tissue some of the rats then were freed of all internal organs and of the entire skin and the head under as clean conditions as possible but not aseptically. The carcass was rinsed under running tap water and cut into pieces. The skin was fixed with the inside up on a wooden board and the subcutaneous fat and connective tissue were collected.

All the tissues were kept frozen, either in an electric freezer at —18 C. or in the carbon dioxide ice box at —76 C., until used. In the latter case, tightly stoppered containers were used to prevent carbon dioxide vapors from coming in contact with the material.

Preparation of Tissue Emulsions.—In some experiments in which relatively small amounts of tissue were involved, the tissues were ground in a mortar with sand, saline solution being added to make a 20 per cent emulsion by weight. This method, however, was found too strenuous for larger amounts, especially for material rich in connective tissue. For larger amounts the following methods were employed:

Heart and Soft Tissues: The tissues were minced and emulsified in a Waring blender of appropriate size. This was usually effected in one-half to three minutes, care being taken to avoid overheating. The suspension was then centrifuged for a short time at low speed to remove coarse materials, which consisted chiefly of

lumps of connective tissue and, when desirable—as in the case of heart—were ground separately in a mortar and added to the original suspension.

Muscle and Connective Tissue: About 200 Gm. of rat carcass devoid of internal organs, head and skin—containing, therefore, chiefly muscle, bones and connective tissue—was cut into pieces and dispersed in the Waring blender to a 50 per cent emulsion in saline solution. The emulsion was centrifuged at low speed. The supernatant was used as "muscle" and was presumably essentially free of connective tissue. The sediment, which consisted of large lumps and threads, was again run in fresh saline solution in the Waring blender and again collected by centrifugation. This process of washing was repeated six to ten times until a grayish white tough fibrous material was obtained. Then this was centrifuged at high speed to obtain it as dry as possible. The yield of this "carcass connective tissue" was roughly about 15 to 20 per cent of the original carcass material.

Grinding of this connective tissue was possible either in the frozen or in the dehydrated state. For grinding in the frozen state, pieces of the material about 5 to 7 mm. in diameter were placed in finely crushed carbon dioxide ice until solidly frozen. The material was then passed through an ordinary hand-driven flour mill which had previously been cooled by the passage of carbon dioxide ice alone. When the temperature of the ground material had risen above freezing, the connective tissue was suspended in saline solution in a mortar. In the more recent experiments, contact of the connective tissue with carbon dioxide ice was avoided because of the possibility that the antigens would be damaged by the concentrated carbon dioxide vapors. Instead of being frozen, the material was dehydrated either by being placed in small pieces in cold acetone, which was changed several times, and subsequently dried, or by being dried from the frozen state by the lyophil process. The dried material was passed once or repeatedly through the flour mill. It was finally suspended in saline solution in a mortar.

Subcutaneous Connective Tissue: The bulk of the fat was removed by placing small pieces of the material into cold acetone, which was changed several times. After drying, it was ground in the flour mill and suspended in saline solution in a mortar.

Preparation of the Streptococci.—Group A beta hemolytic streptococci (mostly of the strain Dochez N. Y. 5) were grown for forty-eight hours on a synthetic nonprotein medium according to the method of Bernheimer and associates,[5] collected by centrifugation, resuspended either in a small volume of the original culture fluid or in distilled water and immediately dried from the frozen state by the lyophil process. The yield was up to 1 Gm. of lyophil-dried organisms per liter of original culture. The organisms were kept in vacuo in the dark in tightly stoppered and paraffin-sealed bottles.

Preparation of Soluble Streptococcic Protein.—It seemed conceivable that when streptococcic protein in solution was admixed to the tissue emulsions rather than whole organisms, better conditions for the formation of antigen combinations between streptococcic and tissue substances might be obtained due to the higher degree of dispersion and the larger surface exposed in the case of soluble streptococcic protein.

For the preparation of streptococcic protein the organisms were ground according to the method of Mudd and others[6] in a low temperature ball mill, simplified for our purpose. As a grinding chamber, a heavy-walled pyrex glass centrifuge bottle

5. Bernheimer, A. W.; Gillman, W.; Hottle, G. A., and Pappenheimer, A. M., Jr.: J. Bact. **43**:495, 1942.

6. Mudd, S.; Shaw, C. H.; Czarnetsky, E. J., and Flosdorf, E. W.: J. Immunol. **32**:483, 1937.

of 250 cc. capacity was employed, which was stoppered by a tight-fitting rubber stopper containing, as an axle, a steel rod which was directly connected with the chuck of a speed-controllable electric motor. The grinding chamber was filled a little over one-half with ⅜ inch (9 mm.) stainless steel balls. The apparatus was mounted with its axle in nearly horizontal position on a laboratory stand, and a tin can of suitable size was used as a "bearing" for the lower end of the grinding chamber, which was immersed in a "methyl cellosolven"–carbon dioxide ice cooling bath (about —75 C.). Lyophil-dried living streptococci in the amount of 2 to 3 Gm. were ground at one time, the ball mill being run at 100 to 150 revolutions per minute for four hours. Then the cooling bath was removed, and after the contents of the chamber had reached a temperature above freezing, 50 cc. of distilled water was placed in the grinding chamber and the ground organisms were suspended by being rotated for a few minutes. The hydrogen ion concentration was brought to p_H 6.8 to 7 with tenth-normal sodium hydroxide. Then the ball mill was allowed to rotate for thirty minutes at room temperature. The suspension was collected with a pipet and there was a second washing with 50 cc. of distilled water. The combined suspension was then immediately cleared by high speed centrifugation in an angle centrifuge, and the supernatant, which contained a considerable amount of protein and which was tested for sterility, was immediately lyophil dried, frozen or admixed to the tissue emulsions and then dehydrated. The ground bacterial sediment, which microscopically was seen to consist of large masses of bacterial debris, also whole cocci, but no or few chains, was discarded.

Preparation of the Final Antigens to be Injected into Animals.—Freezing of the tissue or the tissue-streptococcus emulsions as a means of preserving them until use was highly unsatisfactory because the materials tended to aggregate in large clumps under these conditions. It was therefore found necessary to dehydrate the emulsions after grinding, which was carried out either by the lyophil process or by precipitation and drying with acetone. In a few instances the tissue-streptococcus emulsions were made up immediately before the beginning of the immunization schedule and the material was kept in the refrigerator.

The streptococci were added to the tissue emulsions and well mixed by a short run in the Waring blender. When the emulsion was to be lyophil dried, the streptococci were—previous to their being added to the tissues—killed by adding 10 per cent by volume of ether and letting them stand in the refrigerator overnight. On the other hand, when acetone drying was to be employed, living organisms were admixed to the tissue emulsions, as they were killed by the subsequent treatment. The drying with acetone was carried out as follows: The emulsion was added slowly, under stirring, to three to four times its volume of pure acetone which had been cooled to a temperature of about —50 C. in a bath of carbon dioxide ice in methyl cellosolve or alcohol. After slow rise of the temperature to above freezing, the precipitate was collected by centrifugation, resuspended in acetone, again centrifuged, and so on. After three or four changes of acetone, the precipitate was easily dried under constant stirring at room temperature. While lyophil-dried tissues were kept in vacuo or in a desiccator, this did not seem necessary with acetone-dried materials.

The amounts of the dehydrated materials to be injected were weighed each day and suspended by grinding in a mortar, in distilled water in the case of lyophil-dried tissues or in isotonic solution of sodium chloride in the case of acetone-dried antigens.

Immunization Schedules and Doses.—With rats, as a rule, a schedule of ten intraperitoneal injections made on ten successive days was employed. With the

majority of the animals a second and often a third such schedule was used, intervals of one to two months being allowed between the schedules. In a few instances as many as six schedules were tried.

The standard dose of one schedule was the equivalent of 1 Gm. of fresh tissue (except in the case of connective tissue, in which 0.5 Gm. was employed) and 20 mg. of lyophil-dried streptococci. The soluble streptococcic protein was employed in amounts of 3 to 5 cc. per rat and schedule. Larger doses than this standard dose were not employed, but much smaller ones, about one tenth of the standard dose, were given in some cases. Further, in an attempt to determine the most effective proportion of streptococci to tissue emulsion, the amount of tissue emulsion was reduced to one third and one sixth, the dose of streptococci being kept constant. It seemed, however, from these experiments, that the standard dose and proportion were more effective.

Experimental Groups and the Antigens Employed for Their Immunization.—The following groups of rats were given the antigens listed.

Group 1 (135 rats). Rat heart plus streptococci (strain Dochez N. Y. 5):

(*a*) Rat heart plus streptococci (killed by ether), mixture lyophil dried (43 rats).

(*b*) Rat heart plus streptococci, acetone dried (58 rats).

(*c*) Rat heart, whole, plus soluble streptococcic protein (admixed fresh before each injection, mixture acetone dried) (12 rats).

(*d*) Extract of rat heart (the supernatant resulting from high speed centrifugation of rat heart emulsion) plus soluble streptococcic protein (kept frozen) (12 rats).

(*e*) Rat heart on which streptococci had been grown, acetone dried[7] (10 rats).

Group 2.—Rat carcass connective tissue plus streptococci (68 rats).

(*a*) Rat carcass connective tissue plus streptococci (killed by ether), lyophil dried (32 rats).

(*b*) Rat carcass connective tissue plus streptococci, acetone dried (15 rats).

(*c*) Rat carcass connective tissue, whole, plus soluble streptococcic protein (admixed fresh before injection; mixture acetone dried) (11 rats).

(*d*) Extract of rat carcass connective tissue (supernatant of centrifuged rat carcass connective tissue) plus soluble streptococcic protein (kept frozen) (10 rats).

Group 3. Rat subcutaneous connective tissue plus streptococci (35 rats):

(*a*) Rat subcutaneous connective tissue plus streptococci (killed by ether), lyophil dried (15 rats).

(*b*) Rat subcutaneous connective tissue plus streptococci, acetone dried (10 rats).

(*c*) Rat subcutaneous connective tissue, whole, plus soluble streptococcic protein (admixed fresh before injection) (6 rats).

(*d*) Extract of rat subcutaneous connective tissue (supernatant of centrifuged rat subcutaneous connective tissue) plus soluble streptococcic protein (kept frozen) (4 rats).

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7. Each of 10 rat hearts was ground separately in 5 cc. of isotonic solution of sodium chloride, in a mortar, with the aid of sand. The emulsions were then heavily inoculated with a twelve-hour dextrose-broth culture of streptococci. After the mixture had been incubated for twenty-four hours at 37 C., all samples showed microscopically a fairly heavy growth of the streptococci. The samples were pooled, precipitated and dried with acetone.

Group 4. Rat collagen plus streptococci (20 rats):

(*a*) Rat collagen from rat carcass connective tissue [8] plus streptococci, acetone dried (10 rats).

(*b*) Rat collagen from rat tail tendon [9] plus streptococci, acetone dried (10 rats).

Group 5. The fraction of rat carcass connective tissue which was soluble in 10 per cent sodium chloride solution, plus streptococci. This material represented the pooled extraction fluids from the first four extractions of rat carcass connective tissue with 10 per cent sodium chloride from the preparation of collagen from rat carcass connective tissue (see group 4 *a*) (20 rats).

(*a*) The aforementioned fraction plus streptococci, acetone dried (10 rats).

(*b*) The same fraction plus soluble streptococcic protein, acetone dried (10 rats).

Group 6. Rat skeletal muscle plus streptococci (this material represented the portion of rat carcass which was readily emulsified in the Waring blender; presumably it consisted chiefly of muscle proteins and did not contain an appreciable proportion of connective tissue) (56 rats):

(*a*) Rat muscle plus streptococci (ether killed), lyophil dried (20 rats).

(*b*) Rat muscle plus streptococci, acetone dried (16 rats).

(*c*) Rat muscle, whole, plus soluble streptococcic protein, acetone dried (10 rats).

(*d*) Extract of rat muscle (supernatant of centrifuged rat muscle emulsion) plus soluble streptococcic protein (kept frozen) (10 rats).

Controls.—The following groups of rats as controls were given the materials listed.

Group 7. Rat heart (20 rats):

(*a*) Rat heart, lyophil dried (10 rats).

(*b*) Rat heart, acetone dried (10 rats).

Group 8. Rat carcass connective tissue (20 rats):

(*a*) Rat carcass connective tissue, lyophil dried (10 rats).

(*b*) Rat carcass connective tissue, acetone dried (10 rats).

Group 9. Streptococci (strain Dochez N. Y. 5) (52 rats):

(*a*) Streptococci, ether killed, kept frozen or lyophil dried (10 rats).

(*b*) Streptococci, acetone dried (36 rats).

(*c*) Soluble streptococcic protein (6 rats).

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8. The collagen was prepared as follows: Rat carcass connective tissue was lyophil dried and then ground for about six hours in the low temperature ball mill (in a manner similar to that described for the preparation of soluble streptococcic protein). After being ground, the material was extracted ten times for periods of twenty-four hours each with 10 per cent sodium chloride solution. After the sixth extraction, no protein was demonstrable in the extraction fluid with sulfosalicylic acid. Before admixture of the streptococci, the material was washed with distilled water and then suspended in isotonic solution of sodium chloride.

9. For the preparation of this material, the tendons of rat tails were collected and placed in 0.4 per cent acetic acid. A gelatinous solution of the collagen formed. The noncollagenous materials, which were not dissolved, were removed by centrifugation and gauze filtration.

Group 10. Rat kidney plus streptococci (this group represented animals which had been used for the experiments reported previously in which glomerulonephritis was produced).

Strains of Rats Used.—The majority of the rats employed were derived from the Evans strain, and all of them had been bred in our laboratory. Albino, gray, brown, black and hooded rats were used. The proportion of male rats immunized was slightly greater than that of female animals. Immunization was usually begun when the animals were between 2½ and 4 months old.

A smaller number of rats derived from another strain, namely, the Curtis-Dunning strain, were also used. These were chiefly immunized with rat heart plus streptococci. As will be pointed out later, the results with these animals were especially good. So far, not enough representatives of this strain have been available for use on a larger scale.

The room available for the rats had a relatively high temperature, averaging about 28 C. (82.4 F.). The diet consisted of a commercial dog chow [10] (about 60 per cent of the total) besides grains, bread, cabbage and carrots.

Technic of Serologic Tests.—For serologic studies, the rats were bled from the tail veins. The animals were first placed for a few minutes in a steam bath of about 45 to 55 C. The rat to be bled was then placed in a box from which its tail protruded. The vein of the distal half of the tail was punctured with a 25 gage needle. The flow of blood was kept up by an assistant who lightly massaged the tail in the distal direction. In this manner, 1.5 cc. of blood was relatively easily obtained. When more was taken, e. g., 2 cc., a few cubic centimeters of isotonic solution of sodium chloride was given intraperitoneally immediately after bleeding.

More recently another, more convenient method of bleeding was employed: The rat was etherized in a jar, but only to the point where complete anesthesia just started; i. e., as soon as the animal ceased to move, it was immediately removed from the ether jar. At this early stage of anesthesia the desired amount of blood could rapidly and easily be obtained from the ventral tail vein without any assistance or further manipulation. However, when anesthesia was allowed to proceed further, this satisfactory hyperemia of the tail disappeared, and bleeding was unsuccessful.

Bleeding was usually carried out on the seventh day after the last injection of the immunization schedule. In some rats repeated bleedings were made at intervals of five to seven days to determine the peak of antibody production.

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10. The Ralston Purina Company states that the chow contains the following ingredients: meat meal, dried skim milk, riboflavin concentrate, carotene, cod liver oil, brewers' dried yeast, wheat germ, corn grits, wheat cereal, corn cereal, dried beet pulp, molasses, steamed bone meal, iodized salt.

Chemical analysis shows: protein (digestible) 19 per cent, fat (digestible) 4.7 per cent, fiber 4 per cent, ash 7 per cent, nitrogen-free extract (digestible) 48 per cent, moisture 7 per cent.

Mineral analysis shows:

Iron	0.018%	Sodium	0.67 %
Magnesium	0.09 %	Phosphorus	1.17 %
Silica	0.23 %	Chlorine	0.68 %
Potassium	0.56 %	Calcium	1.5 %

The vitamin content is described by the manufacturers as follows: Vitamin A, about 4,000 U. S. P. units per pound; vitamin B, about 275 Sherman units per pound, supplied by wheat germ; vitamin C, low; vitamin D, about 500 U. S. P. units per pound; vitamin E, supplied by wheat germ; vitamin G, 300 Sherman units per pound.

Because of its high sensitivity, the collodion particle technic was employed for practically all the serologic work.

The antigens used for these tests were extracts obtained in isotonic solution of sodium chloride (0.85 per cent NaCl) from the frozen or freshly ground 20 per cent tissue emulsions, prepared by centrifugation of the emulsions. The supernatants were usually frozen. Freezing did not seem to weaken the activity of the extracts, but it greatly helped to clear them. The extracts for serologic testing were always made from a batch of emulsion made up from a pool of the same tissues from different animals.

Summary of Serologic Results *

Treatment	Rats from Which Serologic Results Were Available	Rats with Low or Moderate Titers (Positive Agglutination in Serum Dilutions up to 1:40 or 1:80)	Rats with High Titers (Positive Agglutination in Serum Dilutions of 1:160 or Higher; up to 1:1,280)	Sum Total of Rats with Autoantibodies (Positive Agglutination in Serum Dilutions of 1:40 or Higher)	Negative
Evans strain rats: rat heart plus streptococci	74	21	19	40	34
Among these: rat heart plus streptococci, acetone dried	22	7	3	10	12
Curtis-Dunning strain rats: rat heart plus streptococci, acetone dried	30	12	14	26	4
Rat subcutaneous connective tissue plus streptococci	25	5	7	12	13
Rat carcass connective tissue plus streptococci	26	6	16	22	4
Rat skeletal muscle plus streptococci	22	8	6	14	8
Streptococci (killed) (tested with rat heart as antigen)	32	1	0	1	31
Rat heart	10	0	0	0	10
Rat carcass connective tissue	10	0	0	0	10

* Recorded is the highest titer observed with the homologous tissue antigen in each rat. With most of the rats serologic tests were carried out repeatedly, i. e., after each immunization schedule.

The tests were set up as follows: Serum dilutions were made from each serum in amounts of 0.5 cc., the doubling dilution being used, usually beginning with a dilution of 1:10. Collodion-antigen mixture (0.2 cc.) was added to each tube. The contents were mixed by shaking, and the tubes were incubated at room temperature for one to two hours. The tubes were then centrifuged in an International Equipment Company centrifuge (horizontally) at 1,400 revolutions per minute for exactly three minutes. The results were read under an artificial light and in front of a black screen while the tubes were carefully shaken, and the sizes of the agglutinations were noted. They were recorded from plus-minus (±) to 4 plus (++++). The collodion particles were prepared as described elsewhere.[11]

11. Cavelti, P. A.: J. Immunol. (*a*) **49**:365, 1944; (*b*) to be published.

For sensitization, the particles were admixed to the antigen immediately before use. In order that nonspecific reactions might be reduced to a minimum, the antigen was diluted to the final dilution (usually 1:15 or 1:20) before addition of the particles. Then enough of the stock collodion was added to make a final dilution of 1:20, and the mixture was ready for being admixed to the serum dilutions.

In most of the tests, about 6 to 10 normal rat serums were run as controls besides serums from rats treated with control antigens, e. g., streptococci alone or tissue emulsions alone. For each batch of collodion particle suspension it was ascertained that the particles alone were not agglutinated by normal or immune rat serum. Antigen controls were set up with saline solution instead of serum.

RESULTS OF THE SEROLOGIC STUDIES

Serologic studies were carried out with a considerable proportion of the experimental animals to determine whether antibodies were formed which reacted with the tissue component of the tissue-streptococcus antigens used for immunization. It should be emphasized that for the in vitro tests, extracts of plain normal tissues were used throughout, i. e., not in combination with streptococci or their products.

Part of the studies were carried out on pools of serums from 5 rats similarly treated. However, the results with pooled serums were markedly inferior to those obtained when individual serums were tested.

Whenever possible, the serologic tests were done not later than twenty-four hours after collection of the serums, as the autoantibodies were found not to be stable when the serums were stored. When serums were retested after a week or two, the titers were considerably lower. However, autoantibodies were still demonstrated in some of the stronger serums after a storage of several months.

Together with the testing of serums from experimental animals, control serums were also tested, which usually were taken from the animals on the same day. These controls comprised samples from normal untreated rats and from rats treated with streptococci alone and tissue emulsion alone. For many of the tests serums were also obtained from the experimental animals before immunization was started, for use as controls.

Some difficulties were encountered regarding nonspecific flocculation of the sensitized collodion particles. This was revealed by flocculation of the antigen in the presence of the control serums. Many of these false reactions could, however, be eliminated in the later tests by an improvement of the collodion technic, described in detail elsewhere.[11b] Some of the tissue antigens proved to be entirely unusable. Collagen, both that from rat carcass connective tissue and that from rat tail tendon, is insoluble in isotonic solution of sodium chloride and thus cannot be tested by the collodion technic. The extract of rat carcass connective tissue in 10 per cent sodium chloride solution was tested in a setup in

which 10 per cent sodium chloride solution was used throughout, also for the serum dilutions; however, the reactions were weak and not considered conclusive. With other antigens and serums known to react positively it was found that 10 per cent sodium chloride solution as a medium greatly inhibited the reactions.

The extracts of carcass connective tissue and of subcutaneous connective tissue in isotonic solution of sodium chloride appeared to yield conclusive results in some large scale tests, as all the control serums were negative, while at other times much nonspecific flocculation was encountered for reasons not yet understood.

Considerably fewer nonspecific reactions were seen with extract of rat skeletal muscle, although here, too, some difficulties were experienced. The most reliable results were obtained with rat heart extract as antigen. However, in the earlier part of the studies, false positive reactions occurred occasionally with serum dilutions up to 1:20. Therefore, in all tests, only reactions with serum dilutions exceeding 1:20 were considered positive.

Generally, as seen in the accompanying table, the results of the serologic studies show that in a considerable proportion of the animals treated with rat tissue emulsions in conjunction with killed streptococci, antibodies were demonstrable which reacted in vitro with extracts of the plain homologous tissue component. The serums from rats treated with either tissue emulsion alone or with killed streptococci alone failed to show any evidence of such antibodies. The titers of the autoantibodies of the tissue-streptococcus treated groups ranged from 1:40 to 1:1,280 (serum dilutions in which positive agglutination appeared). The highest titers were usually found with the samples taken on the seventh day after the last injection. With samples taken subsequently at five to seven day intervals the titers were considerably lower; however, at times autoantibodies were still demonstrable several weeks after immunization. The titers and the incidence of autoantibodies were found to be much higher for the same rats after the second or third schedule of immunization than after the first.

The mode of preparation of the antigenic mixtures appeared to be of considerable importance with regard to the strength of the resulting in vitro reactions. Thus mixtures of tissue emulsion and whole streptococcic cells were the most effective preparations. Although serologically there did not seem to be a definite difference in effectiveness among acetone-precipitated, lyophil-dried or raw mixtures, the use of acetone-precipitated materials seemed preferable in certain other respects, as discussed later. The antigenic effect of mixtures of tissue emulsion and soluble streptococcic protein seemed considerably weaker than that observed when whole streptococcic cells were used. The animals treated

with extracts of tissues (instead of emulsion) in conjunction with soluble streptococcic protein failed to show any evidence of autoantibody production.

The strain of rats appears to be of interest, too, as the representatives of the Curtis-Dunning strain treated with heart-streptococcus antigen had titers far outreaching those of the Evans strain, both in height and in incidence. The agglutinations in the individual tubes were also unusually intense.

In attempts to investigate the specificity of the autoantibodies produced to the various tissues, a considerable number of the serums were tested with heterologous tissue extracts, i. e., with extracts of rat tissues other than the ones used for immunization in each particular case. Thus a number of serums from rats treated with streptococci plus heart, streptococci plus connective tissue, and streptococci plus skeletal muscle were tested with each of these three tissue antigens. As might have been expected, the cross reactions observed were extensive. There was, however, no reaction with any of these serums when they were tested with rat kidney. The serums also failed to react with extracts of rat liver or spleen, while they showed slight reaction with extracts of rat lung.

PRODUCTION OF AUTOANTIBODIES TO HEART IN RABBITS

In the early stage of the studies some rabbits also were immunized with mixtures of killed streptococci and emulsion of perfused rabbit heart. The components were admixed in the proportion of 2 parts of 20 per cent emulsion of heart to 1 part of a suspension of ether-killed streptococci containing 2 per cent by volume of packed streptococcic cells (corresponding to about 4 to 4.5 mg. of lyophil-dried organisms per cubic centimeter). The beginning doses were 1 to 2 cc. of the mixture. The injections were given intraperitoneally either daily or at intervals of three to five days, and the doses were gradually increased to 6 to 7 cc. After every five injections a rest period of several weeks was allowed. The maximum number of injections given was fifteen, administered in three schedules of five injections each. A total of 14 rabbits was so treated. Evidence of production of antibodies reacting in vitro with extracts of plain rabbit heart was obtained. The serums of 7 of these rabbits showed fairly strong reactions in serum dilutions of 1:80 or higher, the serums of 4 rabbits gave weak or doubtful reactions, and those of the remaining 3 were always nonreactive when tested. Some of the strongly reacting serums also reacted strongly with extracts of rabbit skeletal muscle, weakly with rabbit lung and not at all with rabbit kidney, liver, spleen or brain.

COMMENT

The results of these experiments indicate that when rats are given injections of mixtures of killed streptococci and emulsions of certain rat tissues, such as heart, skeletal muscle and connective tissue, antibodies are formed which react in vitro with extracts of the tissue component used for immunization. As such antibodies cannot be produced

by injections of the homologous tissue emulsions alone (i.e., without streptococci), the streptococcus obviously has the capacity to render certain substances of these tissues antigenic for the homologous species, presumably by some loose combination between streptococcic and tissue substances. The antibodies formed have the ability to react with the tissue component alone (i.e., without the presence of streptococcic substances). The tissue components may therefore be spoken of as haptens. From the results reported, these antibodies would logically be termed isoantibodies. However, as will be shown in "Studies on the Pathogenesis of Rheumatic Fever: II," they, or at least some of them, appear to be able to cause tissue damage by means of their reaction in vivo with the tissue of the same animal. The term "autoantibody" is therefore applicable. Formation of such autoantibodies to heart has also been demonstrated in some rabbits which were immunized with killed streptococci plus rabbit heart.

Presumably a multiplicity of autoantibodies are formed by the procedures used in these experiments as well as those reported previously concerning the formation of autoantibodies to kidney. The autoantibodies to heart, skeletal muscle and connective tissue show a great deal of overlapping in vitro. This is not surprising, as connective tissue, for instance, is present in both heart and skeletal muscle; in fact, most of the connective tissue used in these experiments was prepared from skeletal muscle. While the skeletal muscle antigens used were thought to be largely free of connective tissue, the preparations of connective tissue, in turn, could hardly be free of skeletal muscle antigens.

Some of the tissue antigens employed for injection in conjunction with streptococci, such as preparations of collagen and extract in 10 per cent sodium chloride solution of connective tissue, could not be tested serologically by means of the collodion method, as they are insoluble in isotonic solution of sodium chloride. One would probably have to resort to complement-fixation technics for this purpose.

SUMMARY

Rats were given injections of mixtures of killed group A beta hemolytic streptococci and rat heart, rat skeletal muscle and various preparations of rat connective tissue. Autoantibodies to these tissues were formed, which could be demonstrated in vitro with the collodion particle technic and use of extracts of plain homologous tissues.

STUDIES ON THE PATHOGENESIS OF RHEUMATIC FEVER

II. Cardiac Lesions Produced in Rats by Means of Autoantibodies to Heart and Connective Tissue

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IN THE preceding paper [1] ("Studies on the Pathogenesis of Rheumatic Fever: I") evidence has been presented that autoantibodies to heart, connective tissue and skeletal muscle can be produced in rats and rabbits by immunizing the animals with emulsions of the homologous tissues in conjunction with killed streptococci. Streptococcic materials obviously have the ability to render autogenous tissue substances antigenic, thus leading to formation of autoantibodies which react specifically with the respective tissue components. This fact having been ascertained, the question arose whether or not these autoantibodies, by combining with the antigen situated in the tissues in vivo, are capable of acting as a pathogenic agent, leading to damage of these tissues. As already mentioned, these experiments were undertaken with a view to the genesis of rheumatic fever. The organ chiefly examined with respect to such lesions was, therefore, the heart.

METHODS

Most of the materials and methods used in these experiments, such as the tissue and streptococcus antigens and the methods of immunization, have been described in the preceding paper [1] and that description need not be repeated here.

Pathologic Procedure: The rats were killed with ether in groups at various intervals, from seven to one hundred and twenty days, after the immunization schedule, or after various numbers of such schedules, had been carried out. A small portion of the animals died spontaneously after various intervals. The tissues were placed in 4 per cent solution of formaldehyde immediately after the animals had been killed (or as soon as possible after spontaneous death). In most instances the weight of the body, that of the heart and that of both kidneys were recorded. In the earlier part of the experiments the heart was opened and the valves were inspected. However, as the gross changes of the valves were difficult

This investigation was aided by grants from the Commonwealth Fund.

From the George Williams Hooper Foundation for Medical Research and the Division of Medicine of the University of California Medical School.

1. Cavelti, P. A.: Arch. Path., this issue, p. 1.

to evaluate, and especially because much better histologic sections were obtained when the heart was not opened before fixation, this was discontinued. Subsequently, the heart, after being removed from the body, was squeezed between absorbent cotton pads to remove most of the blood from the cardiac chambers and then was placed in solution of formaldehyde U.S.P. After a few days, the heart was cut in half longitudinally in such a way that the mitral and aortic valves were sectioned at the same time. Histologic sections were then made from both halves of the organ.

RESULTS

Gross Pathologic Findings.—With regard to changes referable to the effect of autoantibodies, little of note was seen on gross inspection. In a few instances, slight epicardial deposits of fibrin were visible, chiefly over the auricles and occasionally localized in narrow bands over the upper portions of the ventricles and over the region of the atrioventricular border. Sometimes subepicardial hemorrhages were noted in the region of the ventricles. Some of the hearts appeared to be dilated, especially the auricles, but no reliable standards of reference have been established by which to ascertain this. The cardiac weight, although frequently increased, failed to reveal much with respect to the histologic changes subsequently found. In some of the cases in which the heart was opened, more or less definite verrucous changes were seen in the valves, especially the mitral valve. However, as already mentioned, accurate observations were difficult because of the smallness of the object, and gross inspection of the valves was discontinued prior to fixation in favor of better histologic sections.

Occasional gross changes of other organs were: petechiae and larger hemorrhages of the lungs. This was noted in a number of animals killed relatively early after the immunization schedule had been carried out, i. e., ten to twenty days after the last injection. In other rats the lungs showed spotty pneumonic consolidation and, infrequently as a spontaneous lesion, pulmonary disease with abscess formation.

Enlargement of the spleen was a frequent finding, especially in rats killed relatively soon after treatment.

As to lesions due to the local effect of the injected material, peritoneal inflammatory reaction is to be mentioned. The early stage of this reaction was in the form of fibrin deposit on the peritoneum, with or without a small amount of fluid. Later, adhesions were observed, and in cases in which the injury was severe, abscess formation. In some rats, multiple abscesses, occasionally even with perforation of the abdominal wall, were noted. Cultures of material from these abscesses either proved sterile or revealed contaminating organisms, never streptococci. Peritonitic changes of the sort described were relatively common in rats that had received repeated large doses of raw or lyophilized streptococcus-tissue antigens, especially those which contained a large amount of coarse material and in which the streptococci had been killed by ether.

Such changes, with the exception of slight adhesions, were, however, rare in rats which had been given streptococcus-tissue antigens that had been precipitated and dried with acetone, as well as in those which had been given predominantly soluble materials or fine emulsions. The connective tissue antigens were not obtained sterile and during their preparation further contamination was almost unavoidable. Treatment of these materials with acetone practically sterilized them, which probably accounts to a large extent for the low incidence of peritonitic changes resulting from their use. A further possibility is that the treatment with acetone had a detoxifying effect on the streptococcus and tissue components.

When the incidence of peritonitic changes was compared with that of cardiac lesions in each individual rat, there was no parallelism between the two. There seemed to be rather a slight tendency toward the opposite.

A few rats had abscesses in many organs, including the heart. In some cases these changes seemed to have originated from spontaneous pulmonary lesions; in other instances, from contaminated materials injected intraperitoneally. The lesions of these animals were excluded from the evaluation of the results of the present studies.

Histologic Changes Observed in Heart.—Generally speaking, the lesions to be described involved chiefly the connective tissue structures of the heart, i. e., valves, valve rings and adjacent regions, the parietal subendocardial connective tissue layers, the interstitial and perivascular connective tissue of the myocardium of ventricles and auricles, and the epicardium.

(*a*) Valves and valve rings: Most commonly involved was the mitral valve, somewhat less commonly the aortic valve, often both, and rarely the tricuspid or the pulmonary valve.

The lesions were evidenced chiefly by infiltrative and proliferative changes. Sometimes they were distributed more or less diffusely over most of the valves, but more often their incidence was accentuated in certain areas, such as the tip and other exposed portions of the valve. They also were commonly found in the region of the valve ring. While some of these lesions gave the impression of mere cellular accumulations, others took the form of more or less definite nodular structures.

There was infiltration in which lymphocytes, some leukocytes, monocytes and plasma cells participated. However, a considerable part of the increased cellularity was usually due to fibroblastic proliferation and to the appearance of relatively large cells with a more or less definite tendency toward basophilia. Sometimes the basophilic protoplasm of the latter showed ragged and somewhat indefinite outlines. The nuclei were usually more or less compact and slightly elongated. Pyknotic and fibrocytoid nuclei were, however, sometimes present, and owl-eyed nuclei

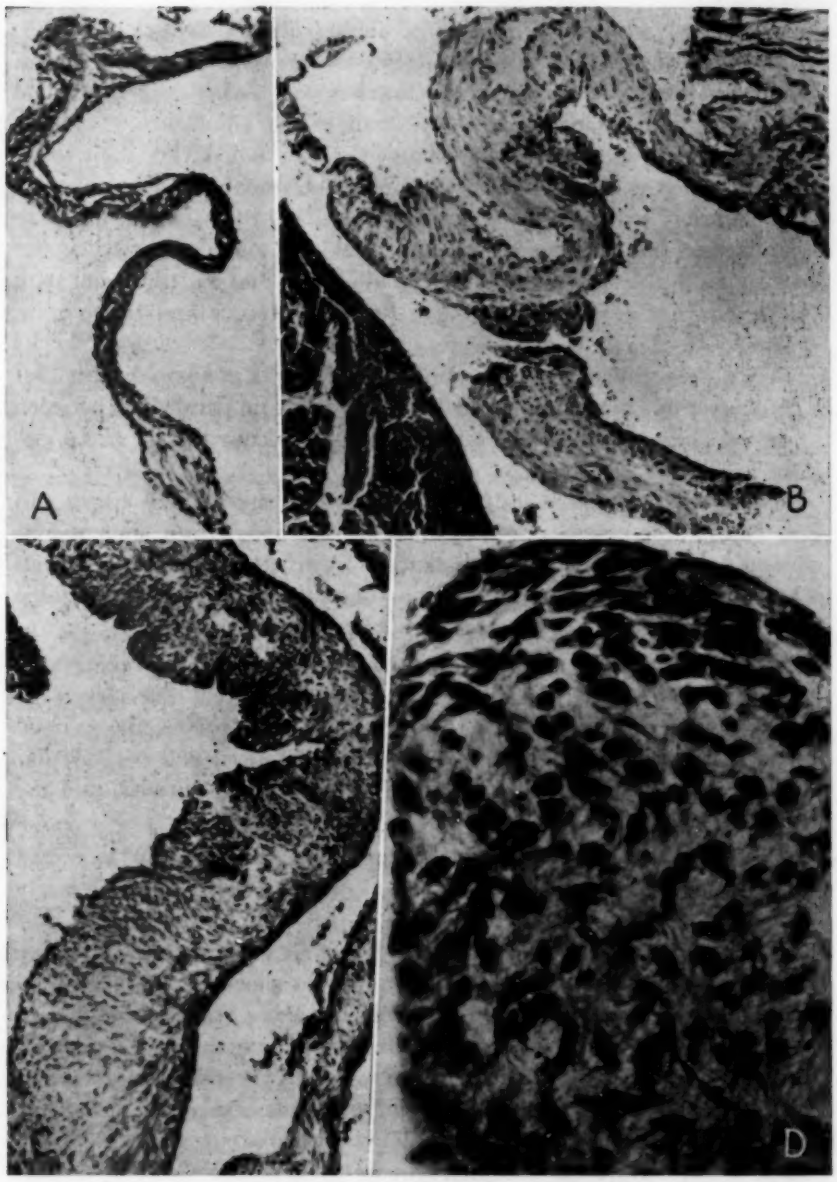

Fig. 1.—*A*, normal mitral valve leaflet of a rat treated with rat skeletal muscle plus streptococci; × 100.

B, inflammatory reaction in the mitral valve of a rat treated with rat carcass connective tissue and streptococci; × 100.

C, inflammatory and proliferative reaction, with considerable thickening, of the mitral valve of a rat treated with rat subcutaneous connective tissue and streptococci; × 100.

D, area of proliferative cellular reaction near the base of the mitral valve of a rat treated with rat heart and streptococci; × 450.

were also noted, though infrequently. Some of the cells of the type described were multinuclear. In a portion of the cases there were apparently degenerative changes of the connective tissue, consisting of swelling and fragmentation of the ground substance (collagen?) with increased eosinophilia. These findings were sometimes associated with more or

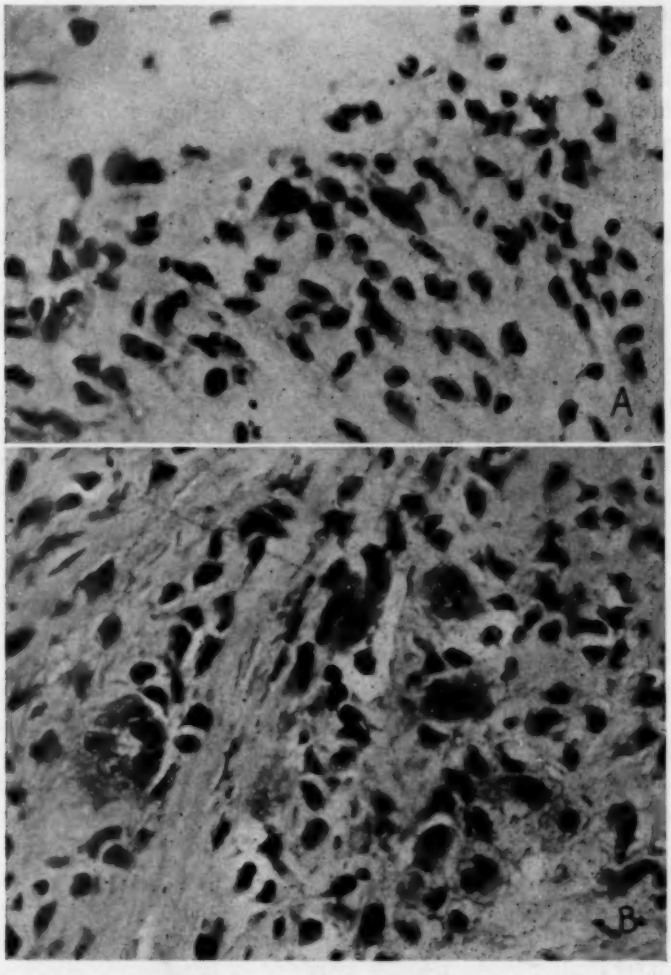

Fig. 2.—*A*, cellular reaction in the valve ring of a rat treated with rat carcass connective tissue and streptococci; × 500.

B, cellular reaction in the valve ring of a rat treated with rat heart plus streptococci; × 500.

less definite cellular nodule formation. As to lesions of the type described, many variations were seen. While granulomatous structures consisting chiefly of somewhat large and occasional multinuclear cells

with a tendency toward basophilia were seen relatively often, it is doubtful whether any of these structures can be considered to resemble closely typical Aschoff bodies. In most of the cases, either the nuclear formation typical of Aschoff cells or conspicuous collagen degeneration was lacking. These lesions were sometimes protruding near the tip of the valves or in other exposed regions besides being located deeper in the valve or in or near the valve ring. Transitions to structures leading to scarring, i. e., with an increased proportion of fibroblasts and fibrocytes, up to lesions made up chiefly of such cells were seen. Some of the valves, therefore, showed more or less definite thickening, either confined to certain portions or involving the whole of a leaflet. In a few cases such thickening was conspicuous. Deformation of the thickened leaflets, although in some cases undoubtedly present, was usually difficult to evaluate from histologic sections only. Vascularization occurred in some of the valves showing considerable proliferative changes. Exudative changes were uncommon. In some cases marked edema of the valves, associated with infiltration, was present. Thrombi were rarely seen on the valves.

(*b*) Parietal endocardium: The changes consisted of edema and thickening of the subendocardial connective tissue layer with varying degrees of inflammatory cell infiltration of this layer. The endocardium itself sometimes showed endothelial proliferation. These changes were not common and were mostly confined to the parietal endocardium of the auricles, especially to that of the left auricle.

(*c*) Myocardium: As already mentioned, the myocardial changes also were predominantly confined to the connective tissue, i. e., the interstitial and perivascular connective tissue. All variations from mere lymphocytic infiltrations to focal and more or less definite nodular lesions of the type described for the valves were noted. In some cases the interstitial and perivascular cellular lesions were impressive and widespread and in later stages associated with a considerable degree of interstitial and perivascular fibrosis resulting in destruction of the adjacent muscle fibers. In some of these instances widespread myocardial scarring, partly with cellular lesions within the scars, was notable. However, on the whole, these myocardial changes were less commonly encountered and less intense than the valvular lesions. When present, the interstitial and perivascular changes were usually more pronounced in the upper portion of the ventricle than near the apex. In some cases they were also outstanding in the auricles.

Involvement of the walls of small arteries and arterioles was not frequently observed. Occasionally there seemed to be some degree of medial infiltration and slight proliferation and hyalinization of the endothelium of small vessels. In some animals more pronounced changes of this sort were noted in the walls of large arteries or in that of the aorta

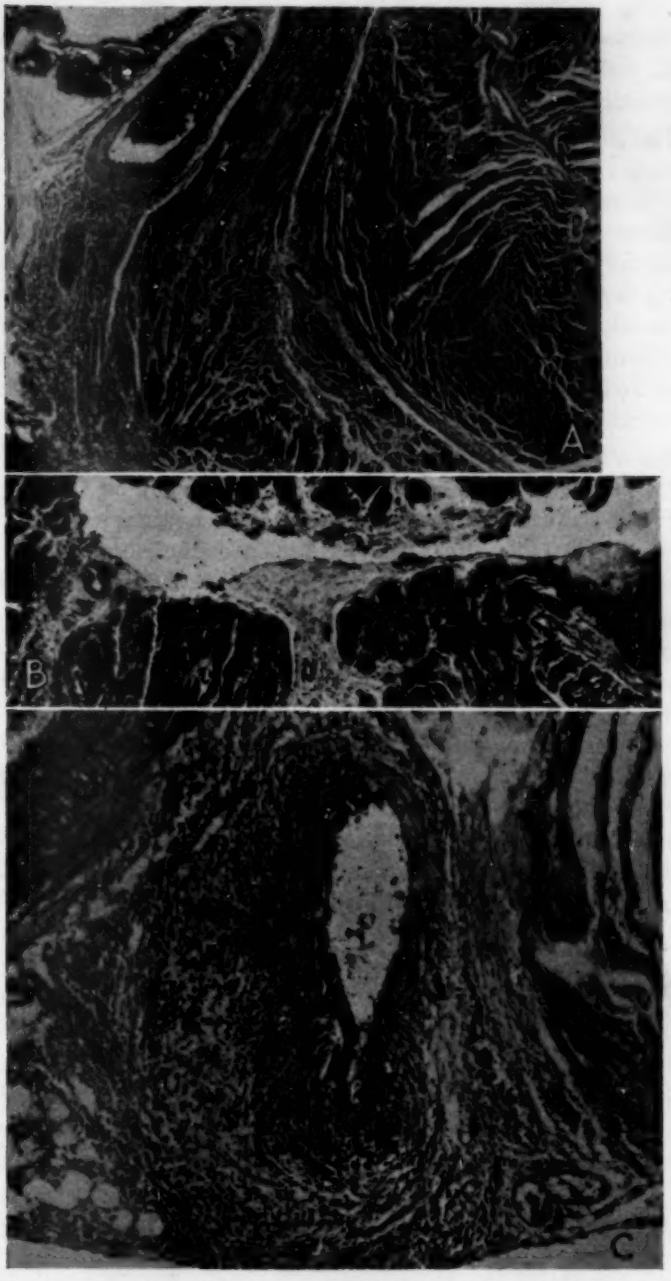

Fig. 3.—*A*, interstitial and perivascular inflammatory reaction and scarring in the upper left ventricular region of a rat treated with rat heart and streptococci; × 50.

B, perivascular scarring in a rat treated with rat heart and streptococci; × 100.

C, arteritis and periarteritis of a medium-sized vessel of a rat treated with rat carcass connective tissue plus soluble streptococci protein; × 100.

near its root, varying in degree up to a striking infiltration of the whole arterial wall with inflammatory cells associated with endarteritis and periarteritic nodule formation.

(*d*) Epicardium: Typical exudative or adhesive pericarditis was not observed, and the pericardial sac was not included in the histologic sections. However, in a few cases the epicardium showed a more or less prominent layer of fibrin with an infiltrate of inflammatory cells. Occasionally small nodular structures were also present. There were varying degrees of organization of the epicarditic layer. These changes were mostly confined to the auricles and were present only in some parts of the auricles; however, in a few instances a more or less continuous epicarditic layer extending around the auricles and a considerable part of the ventricles was noted.

Changes in Other Organs.—Owing to technical circumstances, it was not possible to study other organs histologically on any large scale. However, sections were obtained from lung, liver, spleen, kidney and skeletal muscle (the latter from the region of the elbow) from a small number of rats from each of the major experimental groups.

The most important changes found were perivascular inflammatory reactions. These were common, especially in liver, lung and kidneys, and consisted of collections of inflammatory cells in close proximity to the vessels, particularly arterioles. Some of these collections showed more or less the appearance of small nodules; however, degenerative changes of collagen were not conspicuous, nor were the cells generally of a type comparable to Aschoff cells. Quite common were broad sheaths of inflammatory cells (lymphocytes, plasma cells, monocytes, also some eosinophilic leukocytes) embracing the whole vessel, accompanied in part of the cases by medial infiltration and slight intimal proliferation. Some of these lesions resembled periarteritis nodosa. Sometimes similar sheaths of inflammatory cells were present also around bronchioles in the lungs. In some cases further thickening and slight infiltration of the alveolar wall were seen, and some of the alveoli were occasionally filled with leukocytes and lymphocytes as well as erythrocytes. Slight perivascular reaction was also noted in some of the sections of skeletal muscle but it was not severe or widespread. Interstitial cellular accumulations or nodules were encountered in skeletal muscle in some cases. In a few instances liver and skeletal muscle showed slight perivascular scarring. Sections from spleen generally failed to reveal any significant changes.

Clinical Observations.—Clinical observations revealed little more than the fact that some of the rats, especially during a period of about one to two weeks after the immunization schedules had been carried out, appeared generally sick. Some of these rats died during this period.

No way of estimating clinically the presence and the intensity of cardiac lesions of rats has as yet been developed. Auscultation of the heart failed to yield conclusive findings. Electrocardiograms have recently been taken on some rats, and if the changes prove significant, they are to be reported later.

Arthritis was generally not observed. Routine histologic studies of the joints were, however, not performed. One rat of the Curtis-Dunning strain on which one injection schedule had been carried out with heart-streptococcus antigen precipitated and dried with acetone showed, ten days after the last injection, marked swellings of several joints, especially of the elbows, the knees and the hind feet. The animal appeared very sick, and the swollen joints were obviously painful. The swellings disappeared within about four days. Because of a mishap, the joints were not available for pathologic study. However, the heart of this animal, which was killed because of emaciation two months after the treatment, was one of those showing the most intense changes, especially numerous small interstitial and perivascular scars with and without cellular reaction, further nodular cellular reactions, and thickening of the mitral valve.

EVALUATION OF THE CHANGES OBSERVED IN THE VARIOUS EXPERIMENTAL GROUPS

The types of cardiac lesions were tabulated for each rat, and the intensity of the changes was recorded from plus-minus (±) to 4 plus (++++). Changes which were unquestionably nonspecific and which were also found in normal untreated rats were excluded from this evaluation. The latter changes consisted chiefly of slight lymphocytic infiltrations. It semed desirable to determine, on the one hand, the incidence of all possibly significant lesions, including the slight ones (i. e., those recorded at 1 plus or better) and, on the other hand, the incidence of only the well developed lesions (2 plus or more). The accompanying table shows the percentage incidence of these lesions in the various experimental and control groups. It can be seen that the incidence of cardiac lesions is significant in the groups treated with heart-streptococcus and connective tissue–streptococcus antigens while being negligible in the control groups—whether the evaluation is made on the basis of all, including slight, changes or on the basis of only the more marked ones. The fact that no cardiac lesions resulted from treatment with either streptococci alone or heart or connective tissue alone supports the view that the lesions obtained when these tissues were injected in conjunction with streptococci were due to the autoantibodies found under these conditions. As described in the preceding paper,[1] autoantibodies were obtained essentially only when combinations of streptococci and tissue emulsions were injected, but not on treatment with either of the components alone. The function of the streptococci

obviously is to render autogenous tissue material antigenic. A further strong argument in favor of the pathogenic action of the autoantibodies produced would be to show that there is an interval between immunization and onset of the lesions corresponding to the time necessary for the formation of the autoantibodies. However, as repeated schedules of immunization were carried out on the great majority of the animals, this point could not be readily ascertained. From the little evidence there is, it seems that the changes did not appear before about the seventh to tenth day after the last injection of the primary schedule. A related question would be whether repeated treatment increases the incidence of cardiac lesions. The few rats from which histologic preparations are available after one injection schedule are animals which had died or were killed when very sick apparently as a result of the treatment or its sequences. The incidence of lesions can therefore be expected to be relatively too high in this group. Actually it was about the same as in the group of rats killed after several treatments. The fact, however, emerges from the results that more than three schedules of immunization failed to increase further the incidence of cardiac lesions, although the intensity of the lesions may have increased.

As to the correlation between the titer of autoantibodies and the extent of the cardiac lesions, the results show that there is a fairly good correlation between the incidence and the titer of autoantibodies to heart and to connective tissue when the various experimental groups are compared as a whole. Thus, for instance, rats from the Curtis-Dunning strain responded to heart-streptococcus antigen with much higher titers of autoantibodies and also showed more definite cardiac lesions than the regular strain of rats. Furthermore, the animals treated with soluble streptococcic protein mixed with either heart or connective tissue showed a low degree of serologic reaction and an equally low tendency toward cardiac lesions. However, when the serologic results from individual rats were compared with the histologic ones, the correlation was not so evident in all cases. But this could hardly be expected, as the serologic tests were carried out too infrequently, and in many rats there was an interval of many months between the last serologic test and autopsy.

A noteworthy fact is that cardiac lesions failed to develop in the animals treated with streptococci plus skeletal muscle essentially devoid of connective tissue, although these animals had autoantibodies reacting in vitro with extract of skeletal muscle. This type of autoantibody, therefore, seems to lack pathogenic effects, or it may, owing to the large amount of muscle tissue present in the body, be so highly dispersed that it is insufficient to produce lesions in any one locality.

The evidence available concerning the mechanism of origin of the cardiac lesions produced supports the view that these lesions are due

to the pathogenic effect of the autoantibodies produced when the animals are immunized with autogenous tissues, especially heart and connective tissues, in conjunction with streptococci. An important question arises: In which tissue is the antigen located which incites those autoantibodies that are responsible for the cardiac lesions? From the table it is evident that cardiac lesions were obtained when either heart or connective tissue was used in conjunction with streptococci. In contrast to this, no lesions resulted from the treatment with streptococci plus skeletal muscle largely devoid of connective tissue. The heart antigens used in our experiments undoubtedly contained a large proportion of connective tissue, as care was taken not to lose any of this fraction during the preparation of the antigens. As yet, no animals have been treated with cardiac connective tissue devoid of most of the muscular portion of heart in conjunction with streptococci. However, the results suggest that with the use of heart, too, the connective tissue contained therein may be the site of the antigen in question.

From the results with the two separated fractions of connective tissue (in conjunction with streptococci), i.e., the portion of carcass connective tissue which is soluble in 10 per cent sodium chloride solution, on one hand, and the portion which is insoluble (representing chiefly collagen), together with collagen from rat tail tendon, on the other, nothing definite can be concluded, as the groups are too small and the histologic changes obtained too slight. The fact that the incidence of interstitial myocardial changes was slightly higher in the groups treated with rat kidney plus streptococci than in the other control groups may be explained by the presence of some connective tissue or a related antigen of the kidney. More likely, however, this higher incidence is the result of the inclusion in this group of some very old rats with long-standing chronic glomerulonephritis; in such rats the myocardial lesions may be of a different nature. Also, some of these rats had received exceedingly large amounts and many schedules of injections of streptococcus-kidney antigen, partly also living streptococci. It is conceivable that under such conditions an in vivo formation of streptococcus-tissue antigen might be effected with subsequent production of autoantibodies of the type under study, and consequent cardiac lesions. That in principle the possibility of such mechanisms exists will be shown in another paper.[2]

There did not seem to be any significant difference in the nature of the histologic lesions obtained by treatment with mixtures of either

2. Cavelti, P. A.: Studies on the Pathogenesis of Glomerulonephritis and Rheumatic Fever: In Vivo Activation of Tissue Antigens as a Result of Streptococcic Infection, and Consecutive Formation of Autoantibodies, Arch Path., to be published.

Incidence of Cardiac Lesions

Material Injected	Rats	Percentage Incidence of Histologic Changes													
		Cellular Reaction of Valves		Thickening Scarring of Valves		Cellular Reaction of Interstitial and Perivascular Connective Tissue of Myocardium		Scarring of Interstitial and Perivascular Connective Tissue of Myocardium		Granulomatous Lesions		Pericarditis		Arteritis, Periarteritis, Endarteritis	
		1+	2+	1+	2+	1+	2+	1+	2+	1+	2+	1+	2+	1+	2+
1. Rat heart + streptococci, Curtis-Dunning strain rats	37	51	32	25	16	54	27	8	5	35	11	3	0	3	0
2. Rat heart + streptococci, Evans strain rats	54	45	22	11	7	26	7	13	4	7	0	15	7	17	13
3. Rat heart + soluble streptococcic protein	21	10	0	10	0	10	10	0	0	0	0	0	0	10	5
4. Rat subcutaneous connective tissue + streptococci	31	29	16	20	10	20	16	13	7	10	10	13	10	7	3
5. Rat carcass connective tissue + streptococci	44	46	40	22	18	30	18	20	11	18	13	13	9	9	9
6. Rat carcass connective tissue + soluble streptococcic protein	20	25	10	5	0	0	0	10	0	10	5	0	0	15	10
7. Rat carcass connective tissue, 10% NaCl soluble fraction + streptococci	10	30	20	20	20	20	10	20	20	20	10	0	0	0	0
8. Rat carcass connective tissue, 10% NaCl soluble fraction + soluble streptococcic protein	10	20	10	0	0	10	10	0	0	10	0	0	0	0	0
9. Rat carcass connective tissue collagen + streptococci	10	10	0	20	10	10	0	10	0	0	0	0	0	10	10
10. Rat tail tendon collagen + streptococci	10	10	10	0	0	10	0	10	0	0	0	0	0	0	0
11. Rat skeletal muscle + streptococci	45	4	2	2	2	0	0	0	0	0	0	0	0	2	2
Controls:															
12. Rat kidney + streptococci	57	5	2	3	3	10	2	12	9	3	2	0	0	2	0
13. Streptococci, killed	16	0	0	0	0	0	0	0	0	0	0	0	0	6	6
14. Streptococci, living	10	10	0	10	10	10	0	0	0	0	0	0	0	0	0
15. Rat heart	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16. Rat carcass connective tissue	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total rat heart + streptococci (1+2)	93	46	26	16	11	36	15	11	5	18	5	10	5	11	7
Total rat connective tissue + streptococci (4+5+6)	75	39	29	21	15	25	16	17	9	15	12	13	9	8	7
Total rat heart and rat connective tissue + streptococci (1+2+4+5+6)	168	43	27	18	12	26	16	14	6	17	8	11	6	10	13
Total controls (11+12+13+14+15+16)	148	4	1	3	3	5	1	5	3	1	1	0	0	2	1
Controls, exclusive of rat kidney + streptococci	91	3	1	2	2	1	0	0	0	0	0	0	0	2	0
Controls, treated with tissue or streptococci alone	46	2	0	2	2	2	0	0	0	0	0	0	0	2	0

1+ = slight changes; 2+ = well developed lesions (see page 9).

heart or the various connective tissue antigens and streptococci. Connective tissue is the only component common to all of the tissue antigens which were effective in inciting cardiac lesions. The available evidence therefore suggests that the site of the antigen which incites production of the autoantibodies responsible for these cardiac lesions is the connective tissue.

COMMENT

As described in the first article of this series,[1] autoantibodies to heart and connective tissue are produced in rats immunized with the homologous tissues in conjunction with killed streptococci. The data presented in this paper indicate that the cardiac lesions described are the result of the reaction occurring between these autoantibodies and the corresponding tissues in vivo. Presumably a number of different autoantibodies are formed as a response to injections of materials as crude as mixtures of streptococci and emulsion of heart, connective tissue or skeletal muscle. Evaluation of the changes observed in the various experimental groups, however, suggests that among these the autoantibodies to connective tissue are the ones that are responsible for the cardiac lesions observed, while autoantibodies to skeletal muscle, for instance, apparently have no capacity of pathogenic action. It seems, therefore, that the connective tissue is the site of that antigen which is of greatest interest with respect to the lesions produced.

As compared with analogous experiments concerning autoantibodies to kidney, reported previously,[3] the studies presented here have met with considerably greater difficulties. This was largely due to the fact that no clinical method of estimating the incidence and the intensity of these cardiac lesions was available. Thus, it was not possible to select the animals showing the most pronounced response and to study possibilities of intensifying the lesions by further immunizations in such a selected group. The problem of determining the exact time of onset in relation to the immunization schedule, as well as the life cycle of the lesions, was, for the same reason, rendered rather difficult. On the other hand, the serologic reactions of the autoantibodies to heart, skeletal muscle and connective tissue were, on the whole, considerably stronger than those to kidney reported previously.

It must also be pointed out that not all of the various batches of any one antigen, although they were prepared exactly in the same manner, were equally effective in producing cardiac lesions, a fact which had also been observed in our previous experiments with autoantibodies to kidney. There must, therefore, be a number of unknown factors which greatly influence the immunologic properties of the materials employed.

3. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **39**:148, 1945; **40**:158 and 163, 1945.

The cardiac changes described were not widespread or severe except in a few cases and had thus to be searched for by study of a number of histologic sections of each heart. Because of the smallness of the object, macroscopic inspection failed to yield reliable results, and, therefore, selection of the site of sectioning according to gross findings was not possible. Nevertheless, significant results seem to have been obtained, as shown by comparison of the changes found in the various groups of animals treated with different antigenic preparations.

The experimental glomerulonephritis produced by means of autoantibodies to kidney was, in many cases, of a progressive nature, the renal damage increasing over a period of many months without further injections being made in the animals. In the present studies, with regard to the cardiac lesions, there is no definite evidence of such progressivity except perhaps under conditions of repeated injections of effective preparations of streptococcus-tissue antigens.

As to the question as to what extent the lesions resemble human rheumatic lesions, I do not feel competent to make a definite statement. It can hardly be claimed that typical rheumatic fever has been produced in these experiments. In the cases of more pronounced reaction the structure of the lesions does, however, seem to resemble that seen in cases of rheumatic fever to some degree. More generally speaking, the changes produced are similar to the rheumatic cardiac changes in that they are located in all connective tissue structures of the heart and in that there is a great predominance of valvular lesions. Some degree of fibrotic thickening of the valves as a result of these lesions was seen relatively frequently. However, whether typical valvular heart disease with progressive changes resulting in valvular insufficiency or stenosis and subsequent cardiac decompensation has been produced could not be ascertained and was not obvious. It must further be borne in mind that the heart of a small laboratory animal such as the rat does not contain any large accumulation of connective tissue except perhaps in the valves. The interstitial and perivascular connective tissue of the myocardium is extremely scanty, a fact which might account, perhaps, for the relative infrequency and mildness of the lesions of these sites.

The animals were kept in small cages and in a room of relatively high temperature. Factors such as cold and body activity (fatigue) which are believed to play a role in the genesis of rheumatic fever were thus absent in these experiments.

The fact that changes affecting the valves and other connective tissue structures of the heart have been produced by means of a mechanism which could easily be conceived to operate in human rheumatic fever may be at least as significant as the question whether the experimental lesions are similar to those of rheumatic fever. On the basis of our

results, a working hypothesis of the genesis of human rheumatic fever could be formulated about as follows: During or succeeding the streptococcic infection which precedes a rheumatic attack by about two to three weeks an autogenous antigen is formed by a reaction in which streptococcic substances or products combine with components of the host's tissues, perhaps connective tissue. This antigen incites formation of specific antibodies, which in turn precipitate the rheumatic lesions by reacting in vivo with the antigen situated in the tissues.

From our in vitro studies it would seem that once the formation of autoantibodies has been incited, the streptococcic component is no longer necessary for the ensuing action of these antibodies on the tissues, as our experimental autoantibodies reacted in vitro with the plain tissue component, i.e., in absence of streptococcic material. The hypothesis presented could easily explain the well known and peculiar relationship between streptococcic infection and rheumatic fever, which seems to be an indirect one. The interval between the two would correspond to the time necessary for the formation of the autoantibodies, representing the pathogenic agent of rheumatic fever.

SUMMARY

Rats immunized with combinations of killed streptococci and rat heart or connective tissue form autoantibodies to these tissues, demonstrable in vitro, and, apparently as a result of the pathogenic action of these autoantibodies, undergo changes affecting chiefly the valves and the other connective tissue structures of the heart. In a broad sense these changes may be considered to resemble those of rheumatic fever.

AN ANALYSIS OF CASES OF RADIAL HEMIMELIA

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PEROMELIA

AN EXAMINATION of congenital anomalies of the upper limb suggests that many of the minor deficiencies occurring in the carpal or the digital regions represent a less widespread degree of a more serious longitudinal or ray defect, as distinct from a segmental or annular deformity. Such anomalies (fig. 1), grouped broadly under the heading of peromelia (πηρός, maimed; μέλος, limb), include total absence of the limb (amelia or ectromelia), absence of the forearm and hand (hemimelia), absence of the hand (acheiria or ectrocheiria) and absence of a digit (adactylia or ectrodactylia). In hemimelia the ulnar part of the limb may be intact (radial hemimelia) or less frequently the radial portion may persist (ulnar hemimelia). Furthermore, a middle part may be absent while those portions proximal and distal to it are present, an extreme example of this intercalary peromelia being presence of the shoulder and the hand with absence of the arm and forearm (phocomelia).

RADIAL HEMIMELIA

Radial hemimelia, according to Bunnell,[1] is a deficiency "of the radial bud and, therefore, often includes the carpal and digital rays of the radius and the radial part of the end of the humerus, or the arrangement may be segmental of C.5 and C.6." The thumb is usually absent. In some cases the index finger is involved, because "the middle ray [that of the index] may be involved in abnormalities of either the radial or the ulnar rays" (Forbes[2]) or, according to Bunnell,[1] because the index is included in both the radial and the ulnar buds. Rarely the ulna is duplicated (for example, see Santero[3]). The soft tissues (muscles, arteries and nerves) are commonly affected, and a marked degree of talipomanus valga is usual. The deformity is relatively frequent; Kato[4] collected 253 cases from the literature up to 1923, and since that date

From the Department of Anatomy, King's College, University of Durham.

1. Bunnell, S.: Surgery of Hand, Philadelphia, J. B. Lippincott Company, 1944.
2. Forbes, G.: Anat. Rec. **71**:181, 1938.
3. Santero, N.: Arch. ital. di chir. **43**:173, 1936.
4. Kato, K.: J. Bone & Joint Surg. **6**:589, 1924.

many more instances have been described. It is often hereditary and bilateral, and may be associated with other deformities. Function is considerably impaired, and various types of corrective operations have been devised, one of the most popular being Albee's [5] method of splinting by means of a bone graft.

Pathologic Anatomy.—There is rarely oppportunity of investigating the disposition of the soft tissues in cases of radial hemimelia, and attention is directed chiefly to the bones, both because they may be investigated roentgenographically with ease and because their condition forms the basis for surgical amelioration. It seems somewhat surprising at first sight, therefore, that although an analysis of the osseous arrangement has been presented by Kato [4] and by Kanavel [6] the carpus should

Fig. 1.—Classification of peromelia.

have received inadequate mention. The reason for this is that while the condition of the carpus of the adult can be determined from roentgenograms, in the great majority of the cases of radial hemimelia which have been described the anomaly occurred in an infant less than 1 year old and the disposition of the carpalia is unknown except in those few instances in which dissection was possible.

With a view to elaborating the pathologic anatomy of radial hemimelia and at the same time determining the components of the radial ray of the human upper limb I investigated in the literature 42 limbs in which the radius (or at least its distal part) was absent and in which the carpus was either described or illustrated as a consequence of having been examined by dissection or roentgenography. Two of these 42 limbs

5. Albee, F. H.: Ann. Surg. **87**:105, 1928.

6. Kanavel, A. B.: Arch. Surg. **25**:1 and 282, 1932.

Forty Cases of Radial Hemimelia in Which the Distal Part of the Radius Was Absent

(a, absent; r, reduced; p, present)

		Scaphoid Bone	Trapezium	First Metacarpal Bone	Phalanges of Thumb	Lunate Bone	Trapezoid Bone
Albee [5]	1928	a	a	a	a	p	p
		a	a	a	a	p	p
Bergerhoff, W.: Forschr. a. d. Geb. d. Röntgenstrahlen **36**: 376, 1927	1927	a	a	r	p	p	p
		a	a	(p)	p	p	p
Bertwistle, A. P.: Brit. M. J. **1**: 325, 1923	1923	a	a	a	a	p	p
	1923	a	a	a	a	p	p
Davidson, A. J., and Horwitz, M. T.: J. Bone & Joint Surg. **21**: 462, 1939	1939	a	a	a	a	?	p
Drinnenberg, A.: Ztschr. f. orthop. Chir. **63**: 297, 1935	1935	a	a	a	a	p	p
Fairbank, H. A. T.: Brit. J. Surg. **1**: 553, 1913	1913	?p	?p	a	a	?	?
Fontes, V.: Arq. de anat. **13**: 191, 1929-1930	1930	a	r	r	r	p	r
Forbes [2]	1938	a	a	a	a	p	a
de Freitas Pereira, M. J., and de Lima, J. A. P.: Arq. de anat. **13**: 35, 1929-1930	1930	a	a	a	a	?a	a
Grant, D. N. W.: Mil. Surgeon **69**: 518, 1931	1930	a	a	a	a	p	p
Grant, J. W. G.: Brit. J. Surg. **18**: 166, 1930	1931	a	a	a	a	p	p
Gruber, W.: Virchows Arch. f. path. Anat. **40**: 427, 1867	1867	r	a	a	a	p	p
Haas (Bunnell [1])	1944	a	a	r	r	p	r
Hill, L. L., Jr.: Surg., Gynec. & Obst. **65**: 475, 1937	1937	p	p	p	p	p	p
		p	p	p	p	p	p
Jones, H. W.: J. Anat. **70**: 146, 1936	1936	a	a	a	a	p	p
McWhirter, R. (unpublished)	....	a	a	a	a	p	p
Meckel (Gruber)	1867	a	a	a	a	?	?
		a	a	a	a	?	?
Milne, J. A.: Brit. M. J. **2**: 821, 1915	1915	p	p	p	p	p	p
Schekter, L.: Gaz. d. hôp. **106**: 913, 1933	1933	a	a	a	a	p	p
Silvester (Gruber)	1867	a	a	a	a	?	?
Stoffel, A., and Stempel, E.: Ztschr. f. orthop. Chir. **23**: 1, 1909	1909	a	r	a	r	p	p
	1909	a	a	a	r	p	p
	1909	a	a	a	a	p	p
	1909	a	a	a	a	p	p
	1909	a	a	a	a	p	p
		a	a	a	a	r	p
	1909	a	a	a	a	p	a
		a	a	a	a	p	p
	1909	a	a	a	a	p	p
Tubby, A. *H.*: Deformities, Including Diseases of Bones and Joints, New York, The Macmillan Company, 1912. See Weigel.							
Tyrie: J. Anat. **28**: 411, 1894	1894	a	a	a	a	p	p
Tyrie: Ibid.	1894	p	p	p	p	p	p
		a	a	a	a	p	p
Tyrie	1894	p	p	p	p	p	p
Wakeley, C. P. G.: J. Anat. **65**: 506, 1931	1931	a	a	a	a	a	p
Weigel (Tubby)	1912	a	a	a	a	a	p
Windle, B. C. A.: J. Anat. **36**: 306, 1902	1902	p	p	a	a	a	a

had only one finger each, together with one and no carpale respectively. Out of the remaining 40 the following conditions were collected:

Scaphoid bone	absent in 33 and reduced in 1
Trapezium	absent in 32 and reduced in 2
First metacarpal bone	absent in 32 and reduced in 3
Phalanges of thumb	absent in 30 and reduced in 5

It can be seen, therefore, that each of the bones named may be present in certain cases in which the radius is absent; they are usually missing, however, and were all absent simultaneously in 27 of the 40 cases.

Fig. 2.—Typical carpus in radial hemimelia. For description, see text.

Per contra, a similar investigation of the nearby carpalia in cases of radial hemimelia revealed the following conditions:

Lunate bone	absent in 3 of 35 cases
Trapezoid bone	absent in 4 of 36 cases

Both the lunate and the trapezoid bone were present simultaneously in 30 of 36 cases.

Figure 2 shows the typical condition of the carpus in radial hemimelia; in 17 of the 40 cases the carpus conformed to this representation. The figures listed to this point are illustrated diagrammatically in figure 3, and the individual cases are given in the accompanying table. Sex, age and side of body were recorded too infrequently to be included. If cases of bilateral radial hemimelia had been inserted as cases of unilateral hemimelia 9 cases would have been deducted from the list; this has not been done, however, as the arrangement is by no means necessarily the same on both sides.

There are cases in which the more distal part of the radial ray alone is affected; in 13 such cases the radial styloid process was present

in 2 and absent in 6; in 5 its presence or absence was unrecorded. In the majority of these cases the scaphoid bone, the trapezium and the first metacarpal bone were either absent or reduced, and the phalanges of the thumb were sometimes absent also.

ULNAR HEMIMELIA

It has not been possible to investigate ulnar hemimelia and the constitution of an ulnar ray or rays in a manner similar to that in

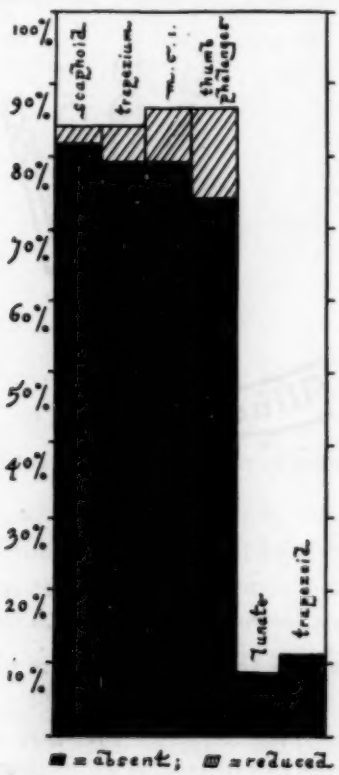

Fig. 3.—Graphic representation of carpalia in 39 cases of radial hemimelia. (This illustration reached the printers before McWhirter's case had been seen.) *M. C. 1* signifies first metacarpal bone.

which the radial deficiency was reviewed, because the condition is rare and because the carpus is more involved, rendering the individual bones which develop more difficult to identify. Some of these cases have been reviewed by Kanavel.[6] Sometimes the entire carpus is absent, but the thumb and usually the index finger are present.

FUNCTIONAL IMPLICATIONS AND ETIOLOGIC CONSIDERATIONS

The applications of these investigations to the functional anatomy of the carpus and the etiology of peromelia have been discussed elsewhere (O'Rahilly [7]).

SUMMARY

A general classification of ray defects of the upper limb is presented.

The osseous anatomy of radial hemimelia is described and the typical condition illustrated. An analysis of recorded cases revealed that in the majority of instances the scaphoid bone, the trapezium, the first metacarpal bone and the phalanges of the thumb are absent while the other skeletal elements of the hand are present. This series of usually interdependent bones constitutes a radial ray.

The composition of the ulnar ray or rays is more difficult to determine, and it has not proved practicable to survey the pathologic anatomy of ulnar hemimelia.

7. O'Rahilly, R.: J. Anat. **80**:179, 1946.

POLYCYSTIC DISEASE OF THE KIDNEY

A Review

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BECAUSE of the mystery that still surrounds its genesis, polycystic disease of the kidneys continues to evoke the curiosity of both pathologist and clinician. It is a disease of paradox, appearing typically either in the newborn or as an affliction of adult life. In the first case it usually leads to the death of the infant without delay, while in the adult it is found to be compatible with a long period of survival, death usually occurring in the second half of life. In either instance the kidneys may be enlarged, in the newborn so much so as to result in dystocia; or, on the contrary, they may be smaller than the normal.

Other kinds of congenital disarrangement are often observed in the infant with cystic kidneys, such as polydactyly, anencephaly or meningocele, and still more important, associated congenital defects of the urinary tract with resulting aplasia of the calices and pelvis, stenosis of the ureters or occlusion of the urethra. Because of this frequent association, the congenital defects of the extrarenal excretory passages have indeed been considered a possible cause of the cyst formation of the kidney and the disease interpreted as a sort of hydronephrosis (Wigand [1]; Marchand [2]). Although from the clinical standpoint it may indeed at times be difficult to differentiate between congenital hydronephrosis and polycystic disease in the newborn, in the adult the distinction can be easily made from the pathologic as well as from the clinical point of view (Bell [3]).

Adult patients with polycystic kidneys usually remain without serious trouble for many years, complaining only of mild lumbar pains that may be aggravated by hard physical work. At times, however, the pains become so unbearable as to suggest renal stone or hydronephrosis and

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1. Wigand, F.: Ueber congenitale Cystennieren (Cystenbildung durch Retention und cystische Degeneration), Inaug. Dissert., Marburg, R. Friedrich, 1899.

2. Marchand, F.: Verhandl. d. deutsch. path. Gesellsch. **2**:187, 1899.

3. Bell, E. T.: Am. J. Path. **11**:373, 1935.

are followed by the voiding of dark brown, blood-stained urine, an episode which is explained by rupture of a cyst wall and opening of the cyst into the pelvis. After many years signs of renal failure appear as they do in progressive Bright's disease, the most important being arteriosclerosis, hypertension, cardiac enlargement, mild retention of nitrogen and sometimes ocular symptoms. A remarkable fact is that in many cases albuminuria is absent. Palpation of bilateral lumbar tumors discloses the nature of the disease, though it should be kept in mind that the left kidney, although enlarged, may not be felt. In these cases pyelography may be useful.

Death usually occurs after a long period of progressive renal failure, but life may be shortened by the rupture of a vessel of the brain or of the meninges. This happened four times in the small series of 7 cases which my associates and I have observed and seems to be linked to small congenital aneurysms of the cerebral arteries (Snapper and Forminje [4]; Crowley and Martland [5]).

The macroscopic features of the polycystic kidney of the adult are so well known as to need no description here; however, it should be remembered that between the typical polycystic form and the so-called solitary cyst of the kidney many intermediate stages may be observed. To diagnose these lesser types of cystic renal disease during the life of the patient is difficult, and so these forms often appear as postmortem discoveries.

Since the macroscopic appearance of polycystic disease is usually so different in the adult and in the newborn, it is not surprising that certain investigators have abandoned the idea that it has the same genesis in both. However, a microscopic study of the condition in both has convinced most that the two forms are best considered as two different stages of a single disease.

MORPHOLOGY OF THE POLYCYSTIC DISEASE

Before summarizing the results of the observations of my colleagues and me, it is necessary to recall what has been contributed to this subject over past years and to examine the different hypotheses that previous workers have offered in explanation of the origin of the cysts.

FACTUAL OBSERVATIONS OF THE PAST

In the case of the newborn it may be easily seen that the majority of the cysts are distended nephrons or enlarged portions of nephrons which are separated from their excretory tubules (Beckmann [6]; Herx-

4. Snapper, J., and Forminje, P.: Acta med. Scandinav. **101**:105, 1939.
5. Crowley, O., and Martland, H., cited by Snapper and Forminje.[4]
6. Beckmann, O.: Virchows Arch. f. path. Anat. **9**:221, 1856.

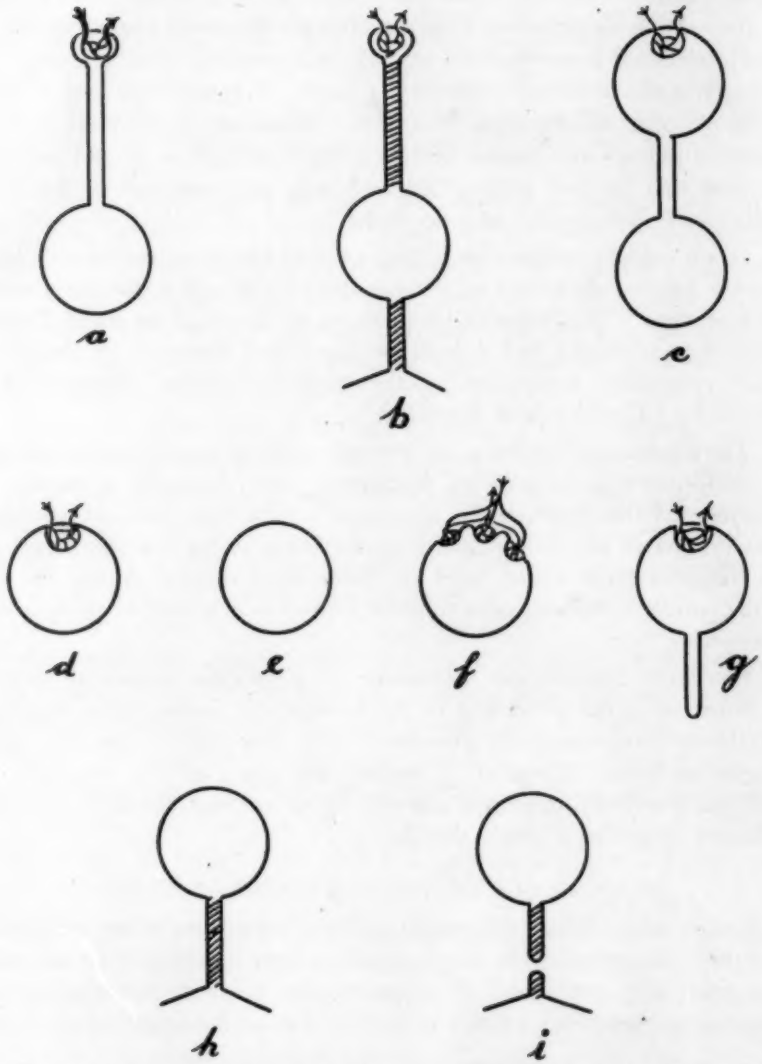

Fig. 1.—Various types of cystic nephrons previously described by other authors: (*a*) Terminal cyst in the course of a closed nephron. (*b*) Cyst described by von Mutach.[11] (*c*) Bicystic nephron. (*d*) Glomerular cyst. (*e*) Cystic vesicle. (*f*) Glomerular cyst with several glomerular tufts. (*g*) Glomerular cyst with blind tubule. (*h*) Excretory cyst opening into the calix. (*i*) Closed excretory cyst.

heimer[7]; Witte[8]; Busse[9]; Berner[10]; von Mutach[11]). In the case of the adult, however, technical difficulties encountered in the reconstruction of the nephrons have prevented in the past a thorough investigation of the topography of the distorted structures.

A brief recapitulation of the various types of cystic nephrons that have been observed in the polycystic kidney of the newborn by previous authors is shown in figure 1.

One type of cyst, first described by Beckmann[6] in 1856, is the so-called glomerular cyst. The glomerular cyst is a closed cavity lined by flattened epithelium. Sometimes a tubule appears to leave the cyst, but when followed in serial sections it is found either to end blindly or to dilate into a terminal sac (Dunger[12]; fig. 1 *c*, *d* and *g*). The vascular tuft of the glomerular cyst is usually well formed but occasionally is poorly differentiated (Busse[9]). Sometimes several tufts of capillaries appear in one cyst, the apparent result of the traction that is made on the original tuft during expansion of the cyst (fig. 1 *f*).

Near the glomerular cysts, tubular cysts may be observed. In many instances the tubular cyst appears to be developed in the distal part of a closed nephron (fig. 1 *a*). Serial sections have shown the tubule to be short and straight, opening at one end into the glomerulus and at the other into the cyst, which is always unipolar. At times the cyst is placed in the middle part of the nephron (fig. 1 *b*), where it may be either unipolar or bipolar: if it is unipolar, the dilatation occurs laterally in the tubule wall, but if it is bipolar, the proximal part of the tubule forms one pole and the distal part forms the other. The further course of this distal portion has been rarely followed carefully. In a case reported by von Mutach[11] it was followed to its opening in the calix, but in this instance the situation was complicated by aplasia of both ureters. We could not find in the literature another report in which cystic nephrons are described as opening into the pelvis, a point the importance of which will appear in the description of our personal observations.

A third type of cyst, described by Ribbert,[13] is the so-called excretory cyst. Cysts of this type usually open into the pelvis, and Berner,[10] by injecting carmine-gelatin into the proximal part of the ureter, demon-

7. Herxheimer, G.: Virchows Arch. f. path. Anat. **185**:52, 1906.

8. Witte, P.: Erworbenes multiloculares Adenokystom und angeborene cystische Entärtung der Nieren, Inaug. Dissert., Königsberg i. Pr., E. Rautenberg, 1896.

9. Busse, O. U.: Virchows Arch. f. path. Anat. **175**:442, 1904.

10. Berner, O.: Die Cystenniere, Jena, G. Fischer, 1913.

11. von Mutach, A.: Virchows Arch. f. path. Anat. **142**:46, 1895.

12. Dunger, R.: Beitr. z. path. Anat. u. z. allg. Path. **35**:445, 1904.

13. Ribbert, H., in Born, G., and others: Bibliotheca medica, Cassel. T. G. Fisher & Co., 1896, pt. C, no. 4.

strated dye in many of them (fig. 1 *h*). A smaller number could not, however, be delineated in this way and were therefore considered by him to be isolated from the pelvis (fig. 1 *i*). The collecting tubules are frequently pinched and distorted by proliferation of connective tissue, which may be abundant in the papillae of polycystic kidneys (Ruckert [14]; Aschoff [15]; Dettmer [16]). And lastly, aglomerular vesicles without any tubular connection are occasionally met in the cortex of the polycystic kidney of the newborn (fig. 1 *e*).

Past investigators have emphasized the abundance of connective tissue that is present in the polycystic kidneys of the newborn. Histologically, it is normal embryonic mesenchyme, which becomes denser around the cysts and is sometimes mixed with muscle fibers (Busse [9]). They have also pointed out that scattered among "round cells" resembling lymphocytes, true isolated epithelial cells can be seen (Busse [9]). Sometimes these are grouped to form small vesicles. Mesenchymatous connective tissue, muscle fibers and isolated epithelial cells are considered to be embryologic remnants because they are normally observed in earlier stages of fetal life.

In rare instances, blood vessels have been said to take part in the cystic process (Berner [10]), a condition which has also been described by Grafflin [17] as occurring normally in a fish kidney. Dyckerhoff [18] expressed the opinion that certain renal cysts have a lymphatic origin since they differ from others by the homogeneous character of their albuminous content and by the absence of luminal casts and epithelial fragments.

Theoretic Conclusions from These Observations.—The most important structural feature of the polycystic kidney of the newborn is that the cystic nephrons are closed systems, not communicating with the pelvis. The only exception, observed in the case of von Mutach,[11] concerns a kidney with ureteral aplasia. For this reason it has been generally agreed that tubular obstruction has something to do with the cystic involvement. This was stated by Virchow [19] as long ago as 1855, who first suggested that tubular casts or insoluble calcium salts might be sufficient to obstruct the urinary flow and so lead to cystic dilatation. Later, however, in 1890, he attributed the formation of

14. Ruckert, A., in Festschrift für Prof. Orth., Berlin, A. Hirschwald, 1903, p. 475.

15. Aschoff, L.: Verhandl. d. deutsch. path. Gesellsch. **7**:69, 1904.

16. Dettmer, H., in Festschrift für Prof. Orth., Berlin, A. Hirschwald, 1903, p. 554.

17. Grafflin, A. W.: Biol. Bull. **72**:247, 1937.

18. Dyckerhoff, K. H.: Virchows Arch. f. path. Anat. **216**:116, 1919.

19. Virchow, R.: Gesammelte Abhandlungen zur wissenschaftlichen Medicin, Frankfurt a. M., Meidinger Sohn u. Comp., 1856.

the cysts to fibrous tissue occlusion of the excretory tubules, which he thought resulted from prenatal inflammation.[20]

This explanation was attacked by Hanau,[21] who held that the cystic transformation of the nephron resulted from congenital aplasia of the medullary tubules. This idea received wider support some years later when embryologists (Schreiner[22]; Felix[23]) showed that the renal tubules develop as the result of a union of two embryologic elements, one of these, the metanephric, forming the glomerulus and the tubule of the nephron, while the other, the ureteric, provided by its dichomatous division the collecting tubules of the medulla. Various processes might conceivably produce a failure of union between the primitive nephrons and the collecting tubules; on account of the frequency of congenital defects of the extrarenal excretory ducts of the polycystic kidney of the newborn, many authors have suggested that aplasia of the intrarenal excretory (collecting) tubules might be the most probable cause of disunion of the two embryologic structures (Dettmer[16]; Ruckert[14]).

According to Busse[9] and Berner,[10] arrested development of the metanephrons results in faulty union of the two embryologic parts of the kidney; in their opinion this conclusion is borne out by the finding of many embryologic remnants in the polycystic kidney of the newborn. More recently McKenna and Kampmeier[24] have given another and somewhat similar explanation of the formation of the cysts. During fetal life, the ureters divide dichotomously to form excretory ducts, which rapidly become attached to nephrons of metanephric origin. However the excretory ducts of the first and second order separate from their nephrons to form the calices, and the nephrons isolated by this process usually atrophy and disappear. The authors suggest that some may persist and become cystic. This suggestion might explain the formation of a few cysts in the central part of the kidney but not the origin of the widely scattered and numerous cysts of polycystic disease.[25]

20. Virchow, R.: Deutsche med. Wchnschr. **36**:11, 1892.

21. Hanau, L.: Ueber congenitale Cystennieren, Inaug. Dissert., Giessen, C. von Münchow, 1890.

22. Schreiner, K.: Ztschr. f. wissensch. Zool. **71**:1, 1902.

23. Felix, W., in Keibel, F., and Mall, F. P.: Handbuch der Entwicklungsgeschichte des Menschen, S. Hirzél, Leipzig, 1910.

24. McKenna, J. M., and Kampmeier, O. F.: J. Urol. **72**:37, 1934.

25. Since this was written, an article by Norris and Tyson (Am. J. Path. **23**: 201, 1947) has called my attention to a publication of theirs which had not reached me during the war years (J. Urol. **46**:147, 1941) in which, after studying serial sections and wax reconstructions of the collecting ducts of polycystic kidneys in 4 cases, they explain the origin of renal cysts by an extension of the normal process of degeneration noted by Kampmeier in the first generation of nephrons to include some or all of the later generations.

Finally must be mentioned the theory, rejected now by most modern investigators, that the formation of cysts is of the nature of a neoplastic process (Brigidi and Severi [26]; Chotinsky [27]; Nauwerck and Hufschmied [28]).

PERSONAL OBSERVATIONS

The personal investigations [29] to be summarized now have been directed toward the solution of the two fundamental problems of the pathogenesis, namely: What structural changes have occurred in the nephrons of the polycystic kidney and what are the functional capabilities of these altered nephrons?

The structural changes were examined by comparing the cysts in the polycystic kidney of the adult with those in the polycystic kidney of the newborn.[29c] Since it is the continuity of the nephron and cysts that is significant, these structures were graphically reconstructed by the method of Peter,[30] and the results of the observations thus made were compared with the findings of previous workers.

The functional capabilities of the nephrons which are so profoundly altered in structure have been examined by determining the composition of the fluid which may be collected from the cysts and also by observing the amounts of a foreign material appearing in the cyst fluid after the material had been injected into the blood.[29 b,d] Inulin was chosen as the test substance because, as shown by Smith,[31] it is excreted only by the glomeruli.

The physiologic examination of the functional capacity of the nephrons of the polycystic kidney as well as the morphologic study of their structure has convinced us that in many instances nephrons with extreme cystic dilatation of their tubules maintain much of the physiologic activity of the normal nephron and that they contribute urine to the excretory process. This conclusion would afford an adequate explanation of the fact that in the polycystic kidney of the adult, at least, renal failure is a late consequence of the disease and

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26. Brigidi, V., and Severi, A.: Sperimentale **46**:1, 1880.

27. Chotinsky, A.: Ueber Cystenniere, Inaug. Dissert., Bern, B. F. Haller, 1882.

28. Nauwerck, C., and Hufschmied, K.: Beitr. z. path. Anat. u. z. allg. Path. **12**:1, 1892.

29. (*a*) Lambert, P. P., and Cambier, P. P.: Beitr. z. path. Anat. u. z. allg. Path. **101**:470, 1938. (*b*) Lambert, P. P., and Muller, P.: Acta med. Scandinav. **101**:338, 1939. (*c*) Lambert P. P.: Le rein polykystique, Paris, Masson & Cie, 1943. (*d*) Muller, P., and Lambert, P. P.: Presse méd. **48**:186, 1940.

30. Peter, K.: Rekonstruktionsmethoden, in Abderhalden, E.: Handbuch der biologischen Arbeitsmethoden, Vienna, Urban & Schwarzenberg, 1921, vol. 9, p. 1.

31. Smith, H. W.: The Physiology of the Kidney, London, Oxford University Press, 1937.

probably linked to the secondary sclerosis that is found only on postmortem examination.

Structural Alterations in the Nephrons of the Polycystic Kidney.—I have had an opportunity to examine in graphic reconstructions 2 cases of polycystic disease of the newborn [32] and 5 cases of polycystic disease of the adult.

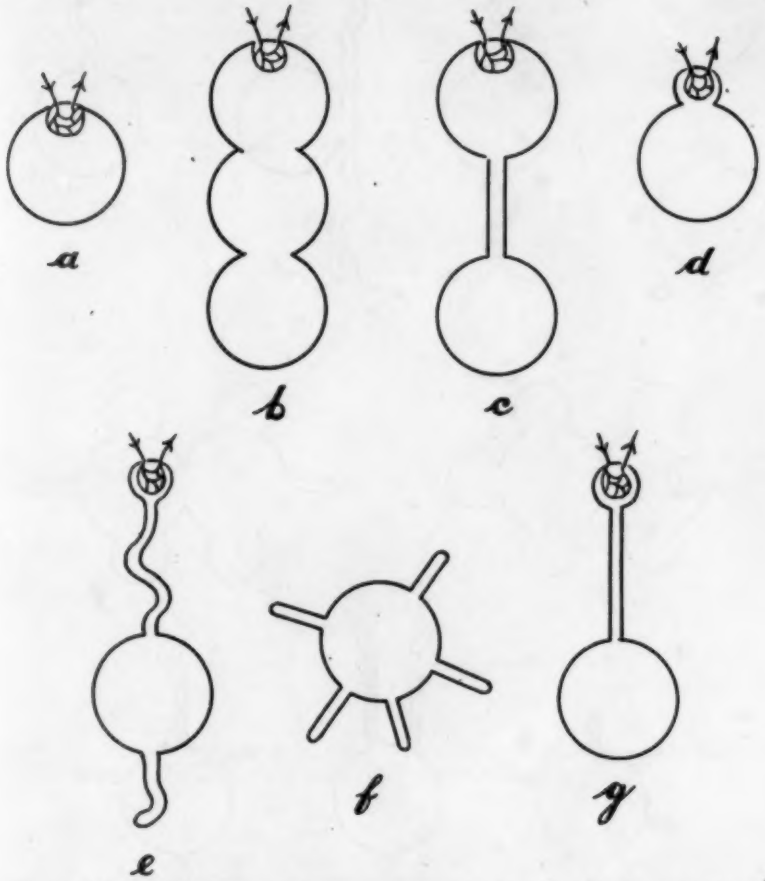

Fig. 2.—The various types of cysts observed in 2 cases of polycystic kidney of the newborn: (*a*) Closed glomerular cyst. (*b*) Closed nephron with several cysts. (*c*) Closed nephron with glomerular and terminal cysts. (*d*) Closed subglomerular cyst. (*e*) Bipolar tubular cyst in the course of a closed nephron. (*f*) Cystic vesicle with blind tubules. (*g*) Terminal cyst in a closed nephron.

(*a*) Polycystic Kidney of the Newborn: Since in the first case of polycystic kidney of the newborn the kidneys present the structural changes seen in most polycystic infantile kidneys, they are described in brief detail.

32. R. de Puysseleyr (J. d'urol. **41**:201, 1936) gave me permission to study this material.

The infant was born dead at 7½ months. Both kidneys were enlarged and so filled with small cysts, averaging 2 mm. in diameter, that on macroscopic examination no normal parenchyma could be seen. The papillae and calices were absent, although there was a narrow pelvis and a patent ureter.

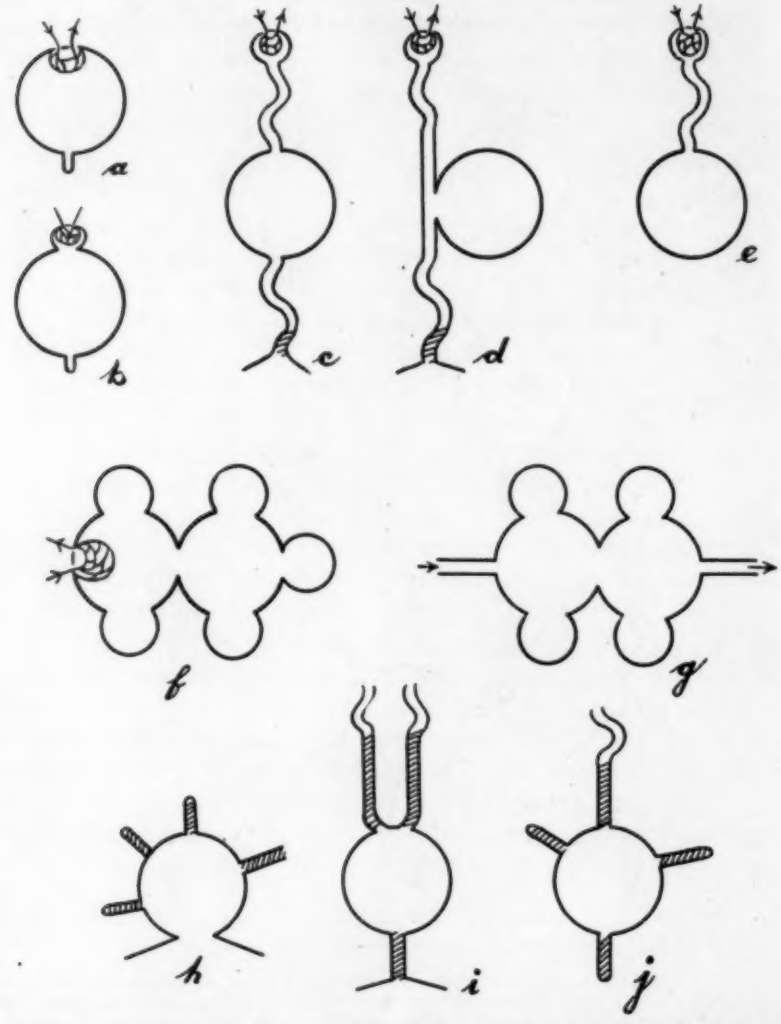

Fig. 3.—Various types of cyst seen in the polycystic kidney of the adult. Shaded portions indicate collecting tubules. (*a*) Glomerular cyst with blind tubule. (*b*) Subglomerular cyst. (*c*) Bipolar cyst in an open nephron. (*d*) Unipolar cyst in an open nephron. (*e*) Terminal cyst in a closed nephron. (*f*) Multilocular glomerular cyst. (*g*) Multilocular tubular cyst. (*h*) Cyst of the calix. (*i*) Tripolar excretory cyst. (*j*) Excretory cyst unconnected with the pelvis.

Microscopic sections showed two distinct layers, a thin external zone formed by the cystic nephrons and a much thicker internal zone formed by irregular cysts and the mesenchymal connective tissue interspersed between them.

The cysts in the external layer were found to be glomerular, subglomerular or tubular. None of them opened into the pelvis (fig. 2 *a* and *b*). The subglomerular type of cyst opened into an adjacent glomerular cavity but did not communicate with a tubule (fig. 2 *d*). The tubular type was either unipolar or bipolar. The unipolar cyst was situated in the distal (fig. 2 *g*) and the bipolar cyst in the middle portion of the nephron, generally at the point where the proximal and the distal convoluted tubule meet (fig. 2 *e*). Their glomerulus appeared normal, and the distal tubule in no case opened into a collecting tubule (fig. 4). Cystic nephrons of the tubular type presented the typical S-shaped form of the embryologic nephron in the second month of fetal life. In one of them (fig. 5) the whole nephron, including the glomerulus, was involved in the cystic change.

The cysts of the internal zone were closed, irregular vesicles. Sometimes blunt short tubes were found to leave the cyst and to pursue a course between

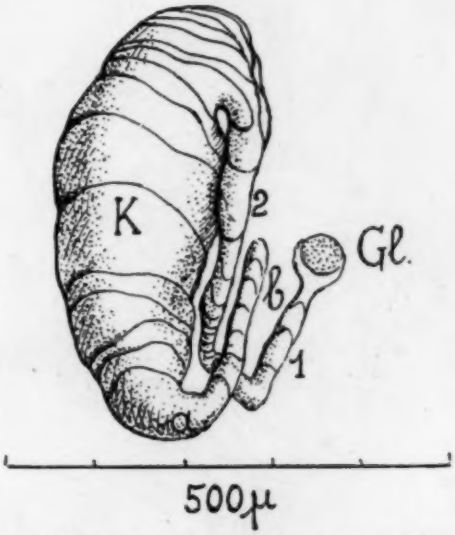

Fig. 4.—Bipolar tubular cyst in the course of a closed nephron. Note the typical S-shaped form of the closed nephron. *Gl* indicates the glomerulus.

neighboring cysts (fig. 2 *f*). What the origin of this type of cyst may be is unknown; it would appear that they are either cystic metanephric vesicles or dilated collecting tubules. The former hypothesis seems the more likely, for if they were cysts of the collecting tubules, one might wonder why they never open into normal excretory ducts or into the pelvis.

Conclusions: The study of serial sections of the kidneys of a premature infant and that of a newborn infant and the preparation of graphic reconstructions of the cystic nephrons added little to what had been already established by previous investigators. The essential fact remains that the cystic nephrons are always separated from their excretory tubules and exist as closed vesicles. It does not follow from this fact that the cyst formation was of necessity the result of a failure

of fusion of the metanephric and ureteric components of the kidney, for it may well be that the cyst formation, taking place in early fetal life, is itself the cause of the disassociation between the two embryologic elements. It seems impossible to prove which of these two hypotheses is the more likely in the light of the 2 cases studied by me though the occurrence of glomerular cystic vesicles which cannot be explained by a failure of fusion of tubules, since no tubule is present, is suggestive that the latter view is more likely correct.

Fig. 5.—Closed polycystic nephron: *C gl* indicates a glomerular cyst; *1, 2, 3* and *4*, tubular cysts. The S-shaped form of the nephron is well shown.

That the failure of union of nephron and excretory tubule is not the decisive factor in cyst formation would be demonstrated if one could find cystic nephrons opening into excretory ducts and therefore able to excrete urine. The only example known in the kidney of the newborn is that described by von Mutach [11] but, as pointed out previously, on account of the bilateral atresia of the ureters, a mechanical factor in cyst formation cannot be excluded in his case.

With a view to following this problem further, the polycystic kidney of the adult will next be described.

(*b*) Polycystic Kidney of the Adult: Since the answer to our problem is concerned with the continuity of the cysts, the tubules and the renal pelvis, it is obvious that it will be answered only by a reconstruction of this continuity. Such a reconstruction of a cystic nephron begins with a careful examination of serial sections of a cyst wall until the opening of the tubule is found; the tubule must then be followed

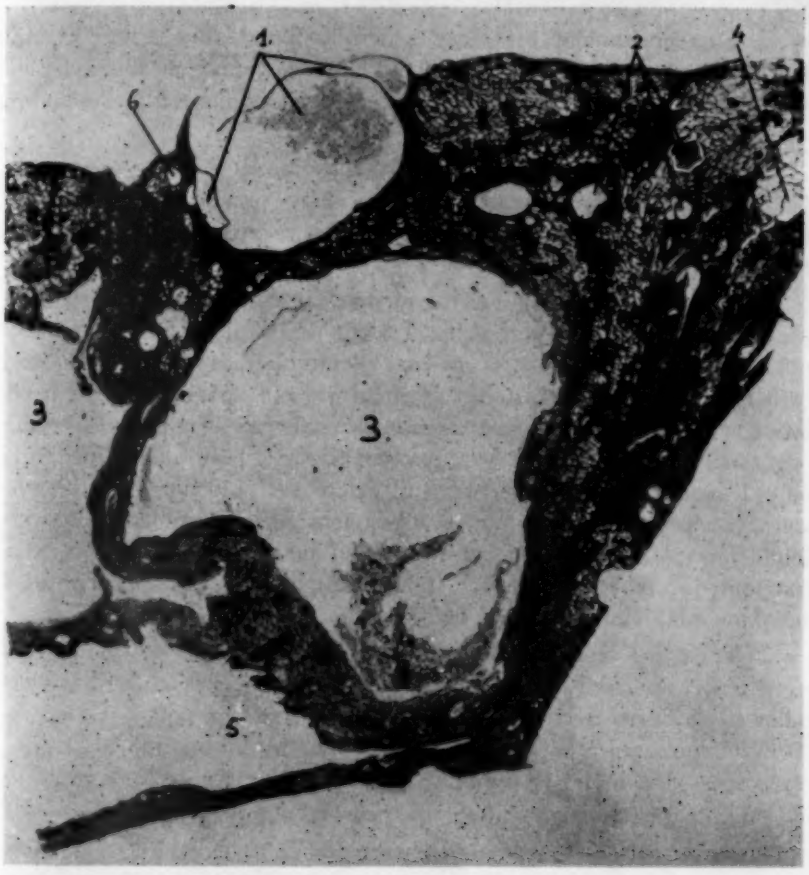

Fig. 6.—Low power microscopic view of the polycystic kidney of the adult: *1* indicates three glomerular cysts; *2*, tubular cysts formed in the course of Henle's loop; *3*, a voluminous excretory cyst; *4*, a multilocular cyst; *5*, part of a calix; *6*, a cyst of the distal convoluted tubule which is seen reconstructed in figure 8.

upward to the glomerulus and downward toward the collecting tubule. To produce a comprehensive picture of what he has seen, the investigator has then to create an objective representation of the cystic cavity and its associated tubules. The Born technic results in a wax model but is so arduous and time-consuming as to be impracticable if any number of nephrons are to be examined, so that in our study Peter's

graphic method was used, a procedure which shows the object in three-dimensional perspective.

A section through the adult polycystic kidney generally shows three types of cysts, which are distributed roughly in three zones (fig. 6).

1. Large, glomerular cysts are found immediately under the renal capsule. They generally contain a small amount of finely distributed granular albuminous material (fig. 6, *1*).

2. Smaller, tubular cysts are generally found at a deeper cortical level. They are as a rule filled with a dense homogeneous coagulum similar to that found in the distal tubules of patients with albuminuria (fig. 6, *2* and *6*).

3. In the medulla a third type, the excretory cyst, is found (fig. 6, *3*). It generally contains a heterogeneous precipitate composed of desquamated epithelial cells, casts and erythrocytes which are held in a fibrinous coagulum.

1. Glomerular Cysts. Twelve glomerular cysts were reconstructed, the diameter of the largest being 1.3 cm. The glomerular tufts were not dissimilar from those of normal glomeruli except that they were rather smaller. Their histologic structure was normal, and their capillaries were fully patent and contained unaltered erythrocytes, which suggests that an effective vascular supply had been maintained. In only 1 cyst was the glomerular tuft sclerosed. Openings in the cyst were occasionally found, but in subsequent serial sections these were found always to lead to a cul-de-sac and never to a patent proximal convoluted tubule (fig. 3 *a*). In 1 example the cyst appeared as a subglomerular closed cavity (fig. 3 *b*).

2. Tubular Cysts. Graphic reconstructions of 10 cysts of this type were made, and in 10 others the cyst walls and their connections were followed in serial sections without actual reconstruction. All of the 20 cysts communicated with the tubular system, a situation in striking contrast to that found in the study of glomerular cysts.

Two distinct types of tubular cysts were observed as follows:

Three of the 20 cysts studied were found to exist as termination of tubules of nephrons which started normally with glomeruli (fig. 3 *e*). In 2 of these instances the site of the cyst was in Henle's loop; in view of the extreme narrowness of the thin descending loop of Henle it is conceivable that even with the perfect series of sections at hand a continuation of the tubule distal from the cyst was missed.

All of the remaining 17 cysts were present in patent tubules; some were unipolar, and each of these appeared, therefore, as a dilatation of the side of a tubule (fig. 3 *d*); others were bipolar, and each of these produced a general ballooning of a tubule (fig. 3 *c*). In every case only one nephron opened into the cyst.

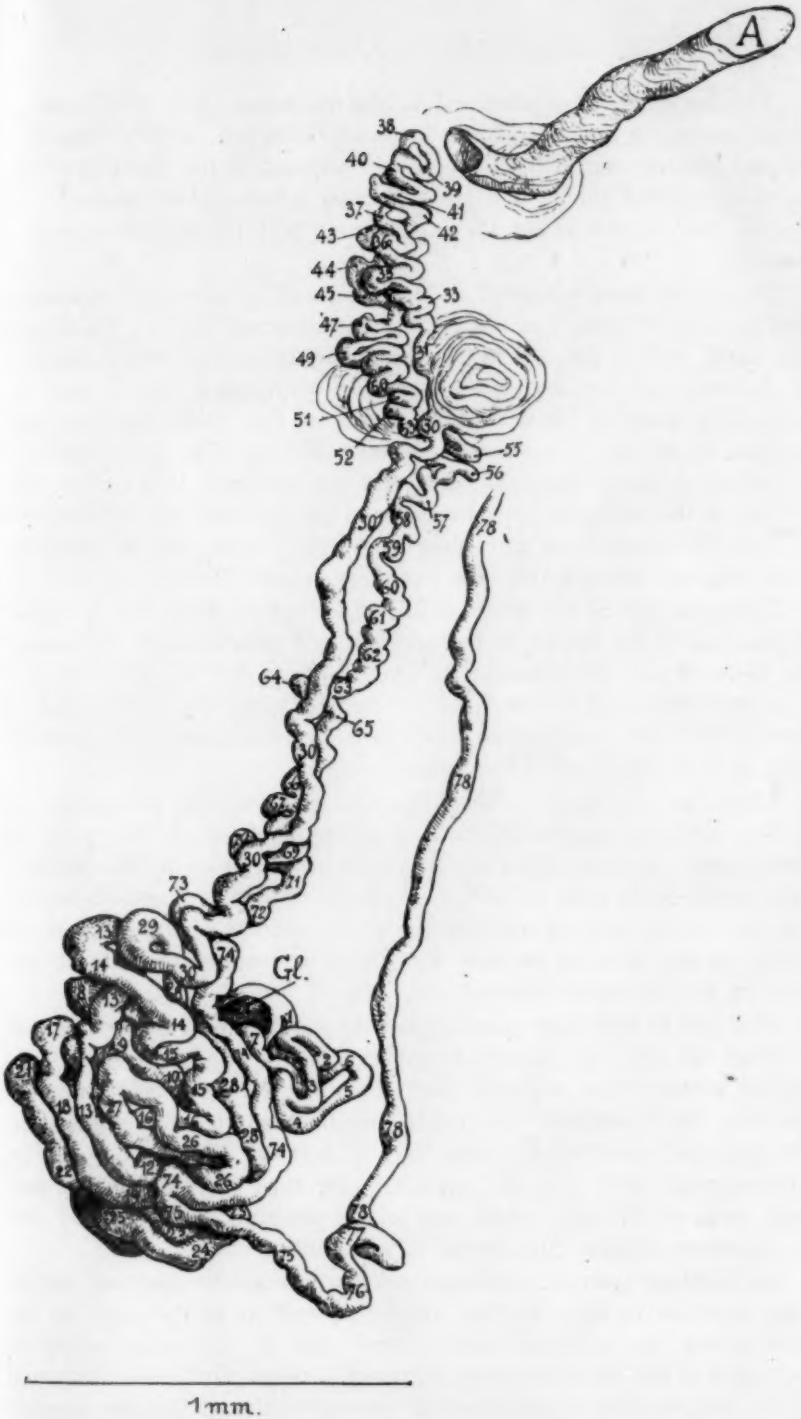

Fig. 7.—Graphic reconstruction of a complete open nephron containing a cyst, the bipolar cavity of which arises in the terminal straight portion of the proximal convoluted tubule (between *30* and *31*). The tubule is reconstructed as far as the collecting tubule (*76, 78*). *Gl* indicates the glomerulus of the nephron; *A*, a lobular artery.

Tubular cysts were observed in the proximal convoluted tubules, in the ascending and descending loops of Henle and in the distal convoluted tubules, and at least 1 cyst was situated at the junction of the distal convoluted tubule and the collecting tubule. Two examples of graphic reconstructions of these structures will illustrate the general findings.

Two cysts were observed in the course of a proximal convoluted tubule. One of them was completely reconstructed (fig. 7). The cyst was small and of the bipolar type and had developed at the junction of the terminal portion of the proximal convoluted tubule and the descending limb of Henle's loop, which in this particular case was tortuous on account of coexistent fibrous sclerosis. The glomerulus and the tubule appeared normal except that the epithelial cells of the first portion of the proximal convolution were flat and without an epithelial brush. No constriction was observed in the course of this nephron, which opened normally into the excretory tubule (*7, 6* in fig. 7).

Two conclusions follow from this observation: first, that a failure of junction of the tubule of the nephron and its excretory component can be ruled out as a cause of the cystic involvement and, second, that it is also impossible to conclude, as Berner [10] did, that any arrest of development had occurred in this cystic nephron, since the different portions of it were everywhere well formed.

Moreover, the cause of the formation of cysts does not appear to be the result of mechanical back pressure imposed on the walls of the tubules. At least there was no apparent occlusion in the nephron as far as its union with the collecting tubule, though it must be admitted that one would have to continue the reconstruction of the latter to its ending in the calix to be sure that there was no obstruction at any point of the excretory channel.

This has in fact been accomplished in the case of a cystic nephron in which the cyst was situated in the terminal portion of the distal convoluted tubule. The nephron was examined from the glomerulus to the calix, but to simplify the drawing we have omitted the glomerulus, the promixal convoluted tubule and a part of Henle's loop. The nephron starts at *1* (fig. 8) just above the turn of Henle's loop, and from there on the more distal part of the nephron, the cyst (*C*) and the excretory tubules are shown in continuity.

In histologic section, abundant protein precipitate and cell débris were observed in the collecting tubule as well as in the calix at the point where the collecting tubule opens into it. At some points in the course of the tubule its lumen narrows, a result which was attributed to the proliferation of surrounding connective tissue, but the passage remains patent all the way to the calix. This observation strongly supports the argument that mechanical obstruction in the lower parts of the excretory tubules is not a factor producing the cyst formation

Fig. 8.—Graphic reconstruction of a cystic nephron including the collecting tubule and the duct of Bellini: *C* indicates the cyst; the asterisks, openings of other collecting tubules into the duct of Bellini; *C ex*, a neighboring tripolar excretory cyst; *C gl*, a glomerular cyst; *C cal*, a voluminous cyst of the calix.

Figure 9 shows a so-called multilocular cyst, an arrangement of confluent cavities, often found in the polycystic kidney of the adult. Cysts of this type have been thought to be true adenomatous formations, but in our opinion they do not differ essentially in their mode of origin from other cysts. Serial sections of 2 examples have been examined. In the first a vascular tuft was found, indicating that it was a glomerular polycystic system; the walls, although carefully examined, did not show an issuing tubule. In the second case four openings were found leading to a single Henle's ascending loop. From each of these four cysts there had developed budding daughter cysts so intimately intermingled that an exact drawing of the resulting complexity was impossible.

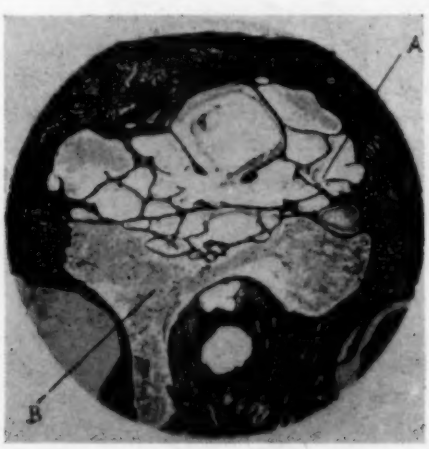

Fig. 9.—Multilocular tubular cyst of the ascending loop of Henle: *A* indicates the proximal portion of the tubule; *B*, the distal portion of Henle's loop.

3. Excretory Cysts. Cysts formed from collecting tubules were commonly found in the medulla. Sometimes, like the cysts of the nephrons, they were bipolar, but characteristically they had three or more poles. This is understandable when the dichotomous origin of the collecting tubules is remembered, for a cyst developing at a junction of two tubules will clearly be tripolar, and if several branches are included within a cyst, it will be multipolar. For the most part, these cystic collecting tubules continued in serial sections as undistended ducts of Bellini to open into the calix. Occasionally, however, the dilated tube was pinched off, leaving the cyst isolated from the calix, a feature already described by Ruckert.[14] Rarely the cyst was at the extreme distal portion of the duct of Bellini, where it opened directly into the calix.

CONCLUSIONS

It is evident from this presentation that though renal cysts of the adult and the newborn are similar in essential structure the cystic organs differ in topography. In both the newborn and the adult the glomerular cysts appear as closed systems; in the adult, cysts occurring along the course of the tubules as a rule are connected with the excretory tubules and the renal pelvis.

For this reason we feel that all cysts must develop independently of such factors as a failure of the tubule of the nephron to unite with an excretory channel or an incomplete development of the tubule of the nephron, and also that they are not the result of fibrous tissue closure of collecting tubules.

It must be admitted that the cause and the genesis of polycystic disease therefore remain unexplained, and all that can at present be said is that the previous conceptions of its origin, based on the three factors which have now been eliminated, are erroneous. At least the detailed and exact morphologic description of the cystic kidney which I have given has opened the way for a physiologic assessment of the functional activity of the "cysts" of the adult kidney that is for so long a time able to maintain life, for I have shown that these cystic nephrons open into the excretory system of the pelvis and the ureter. In the following experiments their functional capabilities were examined.

PHYSIOPATHOLOGY OF RENAL POLYCYSTIC DISEASE OF THE ADULT

In the experiments to be described now the following points were examined: (1) the persistence of glomerular activity in the cystic nephrons, (2) the persistence of tubular activity in these nephrons as concerns their ability to reabsorb water, glucose and urea and (3) the persistence of the ability of the proximal convoluted tubule to store colloidal dyes when these substances are introduced directly into the cystic cavity.

GLOMERULAR FUNCTION OF THE CYSTIC NEPHRON

The persistence of glomerular function was studied by injecting large amounts of inulin into the peritoneal cavities of 2 patients a short time before their death. These patients arrived at the hospital with evidence of submeningeal hemorrhage and died fifteen and ninety-five hours after their admission. Forty-one grams of inulin in 5 per cent solution was injected intraperitoneally in both patients immediately on their arrival. With the first patient it was possible to remove by puncture of the two kidneys 4 samples of cystic fluid before the injection of the inulin; with the second, this was impossible. The 4 samples were found to contain nothing that gave a positive reaction for inulin.

The kidneys of these 2 patients were removed immediately after death in order to avoid postmortem diffusion of the components of the urine. Inulin was found in the fluid of most of the renal cysts of both patients in amounts ranging from 10 to 35 mg. per hundred cubic centimeters, while the blood during the eighteen hours following the injection and preceding death showed around 100 mg. of inulin per hundred cubic centimeters. The detailed findings are shown in table 1.

TABLE 1.—*Amounts of Inulin Appearing in the Fluid of Cysts of Both Kidneys, the Calix and the Bladder After Intraperitoneal Injection of This Substance*

		Urea, Gm. per Liter	Inulin, Gm. per Liter
Case 1	Before injection of inulin		
	Cysts of left kidney	1.53	0.00
	Cysts of left kidney	1.02	0.00
	Cysts of right kidney	1.08	0.00
	Cysts of right kidney	2.11	0.00
	Eighteen hours after injection		
	Cysts of left kidney	1.63	0.00
	Cysts of left kidney	0.64	0.00
	Cysts of right kidney	2.65	0.10
	Calix	7.48	6.20
	Ninety-six hours after injection		
	Cysts of left kidney	1.37	0.25
	Cysts of left kidney	0.91	0.17
	Cysts of left kidney	2.14	0.34
	Cysts of left kidney	1.76	0.16
	Cysts of right kidney	2.73	0.20
	Cysts of right kidney	2.81	±
	Cysts of right kidney	1.76	0.16
	Cysts of right kidney	2.34	0.54
	Cysts of right kidney	1.66	0.17
	Bladder	14.35	1.74
		Creatinine, Gm. per Liter	Inulin, Gm. per Liter
Case 2	Fifteen hours after injection of inulin		
		0.024	Indeterminate
		0.025	Indeterminate
		0.034	0.108
		0.076	0.075
		0.116	Indeterminate
		0.120	±
		0.175	1.101
		0.200	0.144
		0.288	0.147
		0.380	0.290
		0.576	0.160
		0.960	+
		1.020	0.374

It is easy to understand why greater concentrations of inulin did not occur in the fluid of the cysts since the glomerular filtrate formed during the time that elapsed between the injection and the death was diluted by the large amount of fluid already present in the cyst.

As it is generally admitted that inulin reaches the lumen of the tubule only by way of the glomerulus (Smith [31]), one may assume that its presence in the cystic fluid demonstrates persistence of glomerular function. This conclusion was checked by measuring the hydrostatic pressure of the cystic fluid during a surgical operation on a polycystic kidney. The pressure as measured by means of a water manometer varied from 120 to 180 mm., values lower than the filtration pressure

in the glomerulus, which is about 400 mm., so that there is no incompatibility between our chemical data and this physical finding.

TUBULAR REABSORPTION IN THE CYSTIC NEPHRON

Analysis of fluid removed from the cysts immediately after death makes it possible to demonstrate persistence of tubular function in the cystic nephrons. It has been known for several years that the creatinine which has filtered through the glomerulus is not reabsorbed by the tubules, and more recently it has been shown that endogenous creatinine, conversely to the secretion of exogenous creatinine (Shannon and Ranges [33]), is not secreted by the tubules (Winkler and Parra [34]; Miller and Winkler [35]; Steinitz and Türkand [36]). For this reason it is possible, by finding the urine-plasma ratio of endogenous creatinine, to estimate the amount of reabsorption of water that may occur in the cystic nephron. This was done in 2 cases, and table 2 gives the results in detail. The urine-plasma ratio of endogenous creatinine varies from one cyst to another between 0.7 and 36. This means that reabsorption of water took place in many of the cystic nephrons, probably in those parts of the nephrons proximal to the cysts. The occurrence of a urine-plasma ratio lower than 1 on two occasions in the first case may be explained by the fact that during the last hours of life the concentration of the creatinine of the blood increased and the fact that it may take many hours for an equilibrium to be established between the blood and the fluid of the cysts.

Not only is there evidence that water is reabsorbed in the cystic nephrons but it may also be shown that a part of the urea filtered through the glomerulus returns to the blood as it does in the normal tubule. In the same samples of fluid of the cysts the concentration of urea was determined and the urine-plasma ratio of urea established. This varied from one cyst to another between 0.7 and 7. Since the urine-plasma ratio of urea is lower than that of creatinine this can mean only that when reabsorption of water takes place urea is also reabsorbed. It will be observed that in those cysts where the urine-plasma ratio of creatinine is near 1, the urine-plasma ratio of urea is also about 1, a coincidence which indicates that no urea is reabsorbed when no water is reabsorbed. This conclusion agrees with the generally accepted idea that the reabsorption of urea is a passive back diffusion and not the effect of an active tubular process.

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33. Shannon, J. A., and Ranges, H. A.: J. Clin. Investigation **20**:169, 1941.
34. Winkler, A., and Parra, J.: J. Clin. Investigation **16**:869, 1937.
35. Miller, B. F., and Winkler, A. W.: J. Clin. Investigation **17**:31, 1938.
36. Steinitz, K., and Türkand, H.: J. Clin. Investigation **19**:285, 1940.

In figure 10 the reabsorption of the urea of various cysts is compared with the reabsorption of urea observed by Shannon [37] in dogs under different experimental conditions of increasing diuresis. On the vertical axis are plotted the values for

$$\frac{\text{urine-plasma ratio of urea}}{\text{urine-plasma ratio of endogenous creatinine}}$$

and on the base line are plotted the values for the urine-plasma ratio of endogenous creatinine, which varies inversely with the diuresis of the

TABLE 2.—*Concentrations of the Urea and the Creatinine of Samples of the Fluid of the Cysts with the Corresponding Urine-Plasma Ratios*

	Concentration in Fluid of Cysts		Urine Plasma Ratio	
	Creatinine, Gm. per Liter	Urea, Gm. per Liter	Creatinine	Urea
Case 2	0.024	0.53	0.77	0.72
	0.025	0.60	0.80	0.82
	0.034	0.70	1.09	0.95
	0.076	0.76	2.45	1.04
	0.116	0.69	3.74	0.94
	0.120	0.82	3.87	1.12
	0.175	1.14	5.65	1.56
	0.200	1.94	6.45	2.66
	0.288	1.95	9.29	2.67
	0.380	2.21	12.25	3.01
	0.576	2.46	18.50	3.37
	0.960	3.99	31.00	5.46
	1.020	3.06	32.90	4.19
Case 3	0.027	0.85	1.60	1.63
	0.040	0.83	2.35	1.60
	0.041	1.42	2.41	2.73
	0.016	Insufficient	2.72	
	0.058	0.83	3.40	1.60
	0.084	0.83	4.97	1.60
	0.122	1.42	7.18	2.73
	0.153	1.78	9.00	3.42
	0.217	1.48	12.76	2.84
	0.240	1.54	14.10	3.00
	0.245	2.35	14.40	4.52
	0.364	2.85	21.40	5.50
	0.400	3.10	23.50	6.00
	0.556	3.33	32.70	6.40
	0.600	4.15	35.30	8.00
	0.618	3.69	36.40	7.13

dog, according to the data, and is proportional to the reabsorption of the water of the cyst fluid. The results obtained in these two figures are seen to be similar.

Glucose has been found in small amounts in all samples of cyst fluid that have been examined, but the urine-plasma ratio for glucose was lower than 1 in 3 instances and lower than 1.3 in every case in which it was determined. When phlorhizin sufficient to induce glucosuria was injected into a patient twenty-four hours before an operation on the right kidney, the urine-plasma ratio for glucose was found higher than 1.3 in 12 of the 14 samples of cyst fluids removed during the operation (table 3).

37. Shannon, J. A.: Am. J. Physiol. **122**:782, 1938.

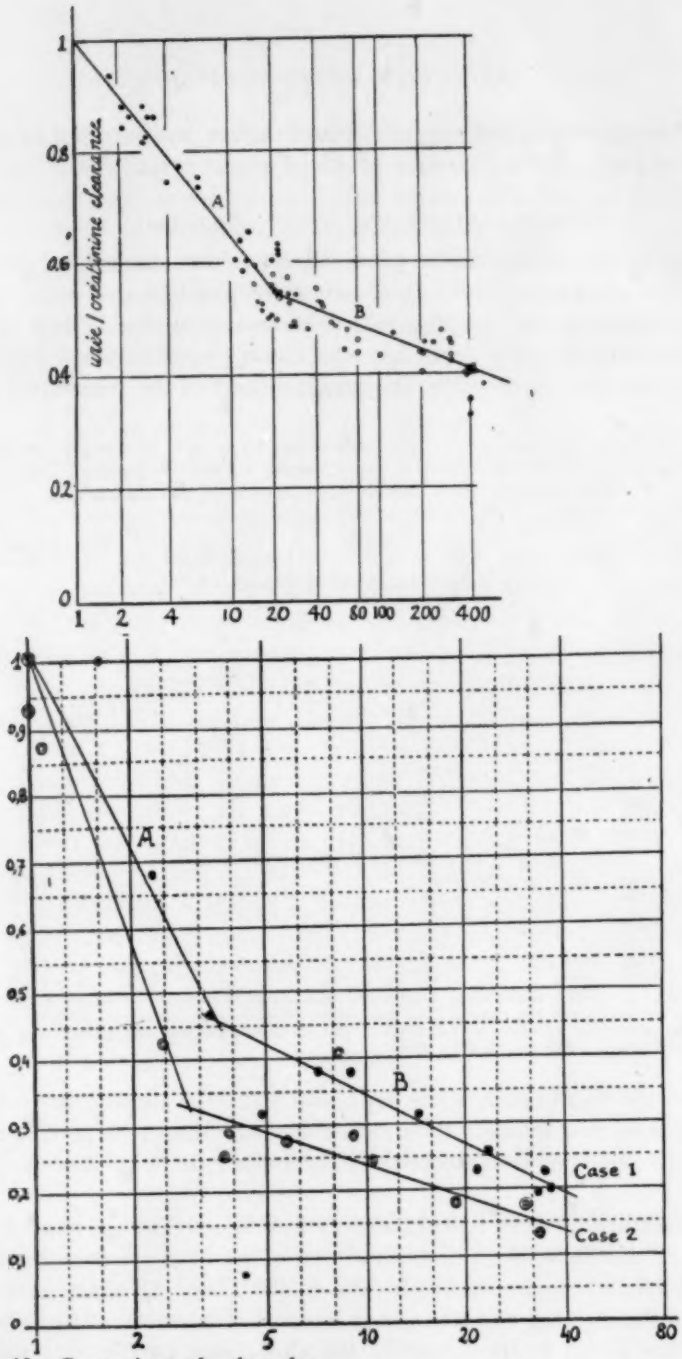

Fig. 10.—Comparison of values for $\frac{\text{urine-plasma ratio of urea}}{\text{urine-plasma ratio of creatinine}}$ at different levels of water reabsorption. The data in the upper figure are taken from Shannon [37] and were obtained from dogs at a time when forced diuresis had been induced and at a time when the output of urine was smaller. The data in the lower figure were obtained from the study of samples of fluid removed from cysts in 2 cases of polycystic kidney. In both figures the urine-plasma ratios of creatinine (horizontal axis) measured the amounts of water reabsorbed by the tubules.

These results indicate persistence of active reabsorption of glucose in those parts of the nephrons proximal to the cysts.

TUBULAR ABILITY TO STORE COLLOIDAL DYES

Finally the ability of the proximal convoluted tubules of the cystic nephrons to store colloidal dyes such as trypan blue and india ink has been examined. In studying this phenomenon in the kidney of Salamandra maculata some years ago with Gerard and Cordier [38] I observed that trypan blue is stored in the proximal part of the convoluted tubule

TABLE 3.—*Concentrations of the Glucose and the Creatinine of Samples of the Fluid of Cysts Taken from the Right Kidney During Phlorhizin-Induced Glycosuria and from the Left Kidney Six Months Later (Case 3)*

Creatinine, Gm. per Liter	Urine-Plasma Ratio of Creatinine	Glucose, Gm. per Liter	Urine-Plasma Ratio of Glucose
	Right Kidney: After Phlorhizin *		
0.040	2.35	1.17	1.42
0.041	2.43	1.56	1.90
0.046	2.72	1.86	2.27
0.057	3.35	Insufficient	Insufficient
0.084	4.97	0.97	1.18
0.122	7.18	0.76	0.93
0.153	9.00	2.53	3.08
0.217	12.76	Insufficient	Insufficient
0.240	14.10	0.92	1.12
0.245	14.40	1.69	2.06
0.364	21.40	1.14	1.40
0.400	23.50	1.55	1.88
0.556	32.70	1.66	2.02
0.600	35.30	1.82	2.22
0.618	36.40	1.79	2.18
	Left Kidney: Without Phlorhizin		
0.021	1.23	0.62	0.750
0.022	1.30	0.21	0.250
0.025	1.47	0.28	0.340
0.028	1.64	1.10	1.340
0.029	1.70	1.09	1.330
0.030	1.76	1.01	1.230
0.069	2.30	0.13	0.150

* The samples from the right kidney were collected during an operation performed twenty-four hours after the injection of phlorhizin. The samples from the left kidney were removed immediately after death six months later.

next to the glomerulus, while india ink is stored more distally in the same convoluted tubule. This difference was shown to be due to the fact that india ink is composed of larger-sized particles than is trypan blue.

A mixture of these two substances was injected into a cyst by renal puncture shortly before the death of one of the aforementioned patients. By a fortunate circumstance the dye in the lumen of a nephron opening into the injected cyst was found in serial sections.[29a] Although both dyes were found in the lumen of the glomerular part of this nephron proximal to the cyst, only trypan blue was stored in the cells at this level of the tubule, while in the more distal part of the tubule cellular

38. Gerard, P., and Cordier, R.: Arch. de biol. **43**:367, 1932; Biol. Rev. **9**:110, 1934. Lambert, P. P.: Arch. de biol. **47**:125, 1936.

storage was limited to india ink. This finding reproduces exactly what was observed in the kidneys of the salamander when the dyes were introduced into the lumens of nephrons which open into the peritoneal cavity by means of nephrostomes. So far as we know this is the only instance on record in which tubular cells of the human kidneys stored a substance with as large particles as india ink, for normally india ink does not reach the lumen of the tubule because it cannot pass through the glomerular membrane.

CONCLUSIONS

From these experiments it appears that the cystic nephrons of the adult retain a considerable part of their functional ability, and there is no reason to doubt that they take part in the formation of urine.

This cannot be true of the cystic kidney of the newborn. Although the function of the cystic nephrons of infantile kidneys has not been examined by experiments similar to those applied to the cystic nephrons of adult kidneys, the nephrons have been carefully examined by morphologic methods and in the observations so far presented appear as closed systems, isolated from the pelvis of the kidney. As a result, the infant dies promptly, often before birth, while the adult survives, often to late middle age. Our observations answer satisfactorily a question that frequently arises when one is considering the last stages of polycystic disease in the adult, namely: Where is the functioning renal parenchyma, and where is the urine coming from? As shown in the foregoing pages, the cystic open nephrons are able to provide for the formation of urine to a certain extent and this function they doubtless maintain in the last period of life when the neighboring noncystic parenchyma has been destroyed either by the enlarging cysts or by the development of sclerotic tissue.

SUMMARY

Graphic reconstructions of polycystic kidneys of the newborn have shown that the cysts are closed cavities consisting of distended nephrons which are isolated from the pelvis, a conclusion which agrees with the opinion of previous investigators.

The same method of examination applied to the adult type of the polycystic disease showed glomerular cysts, which appeared as closed cavities, and tubular cysts, which had developed in the course of the nephrons.

When the cystic nephrons were examined in serial sections and in their reconstructed continuity it was found that most of them were completely developed and connected in the usual way with the collecting tubules and that these opened freely into the pelvis.

The tubular cyst appeared in the course of the proximal convoluted tubules, in Henle's loops, in the distal convoluted tubules and also in

the medulla along the course of the collecting tubules. From the morphologic point of view it therefore seemed that they might well have retained a part of their renal function. This was investigated by physiologic methods.

Inulin appeared in the cysts after intraperitoneal injection, an indication of glomerular filtration. The fluid from the cystic tubules was found to have the essential characteristics of the urine. Its creatinine level was typically many times higher than that observed in the blood, an indication that water was being absorbed by the cystic nephrons. The same observation was found to be true of urea. Moreover, a back diffusion of urea in the cystic nephrons similar to that which occurs in the normal nephron could be demonstrated. Although the fluid of the cysts contained small amounts of glucose, the concentration of this component increased after the injection of phlorhizin.

And lastly, colloidal substances injected directly into the cyst were stored in the cells of the convoluted tubule showing that this peculiar function of the proximal convoluted tubule had persisted in the cystic nephron.

BOVINE LEPTOSPIROSIS

Pathologic Observations on Experimentally Infected Calves

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RECENTLY it was shown that a disease of cattle of Palestine, characterized by fever and, when severe, by jaundice, was caused by a Leptospira which differs serologically and in its pathogenicity from Leptospira icterohaemorrhagica and Leptospira canicola.[1] Cases of severe leptospirosis of man caused by the same strain were also reported.[2] The disease was successfully transmitted to calves through several passages, and the clinical picture has been reported, together with the changes in the blood and the urine of infected animals. The pathologic-anatomic findings in this series of animals and in additional animals of later experiments are the subject of the present study.

REVIEW OF THE LITERATURE

Knowledge of leptospirosis of cattle is of relatively recent date, and literature on the pathologic aspects of this disease is scarce.

Jungherr [3] described observations made on 3 young cows dying of a "mysterious" disease which at autopsy revealed lesions similar to those seen by the same author in cases of canine leptospirosis. The diagnosis was supported by the histologic demonstration of "leptospira-like bodies" in 3 cases, in the kidneys, in a liver and in a mesenteric lymph node. Marsh [4] succeeded in demonstrating leptospiras in histologic sections of liver and kidney taken from a calf which died during an epidemic of icterohemoglobinuria. In his report the histologic changes were only briefly mentioned. More recently, Mathews [5]

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1. Bernkopf, H.: Harefuah **30**:110, 1946. Bernkopf, H.; Olitzky, L., and Stuczinski, L. A.: J. Infect. Dis. **80**:53, 1947.

2. Padersky, B.: Harefuah **30**:117, 1946. Btesh, S.: ibid. **30**:121, 1946. Ephrati, P.: ibid. **30**:125, 1946.

3. Jungherr, E.: J. Am. Vet. M. A. **105**:661, 1944.

4. Marsh, H.: J. Am. Vet. M. A. **107**:119, 1945.

5. Mathews, F. P.: Am. J. Vet. Research **7**:78, 1946.

reported on the necropsies of cattle dying during a field epidemic. The author found leptospira-like organisms in sections of liver and kidneys and recognized a certain similarity between the histologic changes in cattle and those in dogs with leptospirosis. He hesitated, however, completely to accept the etiologic role of leptospiras, because of excessive variations in the clinical picture, which in its acute-fatal, moderate and chronic forms conformed neither to the intensity of the damage of tissues nor to the number of leptospiras in the tissues. The author attempted to transmit the disease by subcutaneous injections of blood and of emulsions of kidney and liver from diseased animals. A febrile disease was thus produced in 7 calves, but the animals were not killed for pathologic examination. Transmission experiments in which sheep and goats were used gave negative results, while two guinea pigs became ill, but no characteristic lesions of tissues developed, and no spirochetes could be demonstrated.

An earlier and extensive study of the anatomic findings in bovine leptospirosis was published by Avrorow,[6] in 1941, but unfortunately only an abstract of this author's paper was available to us. During an epidemic occurring in the Caucasus he examined more than 40 field cases of leptospiral jaundice, as well as 4 experimentally infected calves. The lesions in the latter were reported to conform with those in the spontaneous cases, and spirochetes were demonstrated in histologic sections of several organs.

MATERIAL STUDIED

Twelve calves were available for examination in the present study. At the time of infection the animals were about 10 to 14 days of age. One of them (calf 44) had been experimentally infected with blood taken from a cow at the beginning of a "spontaneous" attack of jaundice, in the preicteric stage, and 8 calves were subsequently infected in consecutive passages. Three further animals were inoculated with a pure culture of the strain of leptospiras which was isolated from the blood of a human victim of the disease and which behaved serologically and in its pathogenicity exactly as did the strain isolated from infected calves of the first series of experiments.[7]

At necropsy, pieces of liver, spleen and kidney were fixed for histologic examination in Zenker's fluid as well as in 10 per cent formaldehyde solution. Sections were stained with Ehrlich's hematoxylin-eosin, iron hematoxylin-Van Gieson, Heidenhain's azan, Foot's silver impregnation or sudan III; blocks of liver and kidney were impregnated with silver according to Levaditi's method.

Bacteriologic cultures were made from the blood and organs, and leptospiras were isolated from the blood of several animals. Paratyphoid-B bacteria were isolated from the organs of 4 animals and were found to belong to four serologically different groups. In no instance had this infection, which is rather common in Palestinian cattle, been suspected on clinical grounds. The presence of blood parasites was excluded by the examination of smears from blood and hemopoietic organs.

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6. Avrorow, A. A.: Ztschr. f. Veterinärk. **53**:32, 1941.

7. Bernkopf, H.; Stuczinski, L. A.; Gottlieb, T., and Halevy-Katz, C.: To be published.

FINDINGS

The infections of our animals did not run a uniform course. Three separate clinical pictures could be differentiated, and our material was accordingly grouped. The first group included 6 animals (3 from the blood passage series and 3 infected with a pure culture of the strain of leptospiras studied) in which fatal jaundice developed. To the second group belong 2 animals which died with no jaundice developing. Four animals in which a mild disease developed and which recovered constitute a third group. Additional details are given in each section.

GROUP I.—These animals died of the fulminating form of the disease, which was accompanied by jaundice (calves 44, 45, 55, 95, 96 and 106).

Summary of Clinical Observations.—Calf 44 was inoculated with blood taken from a diseased cow in the preicteric stage. The calf had jaundice ten days after inoculation and died on the eleventh day. Blood from this animal was transmitted to calves 45 and 52, and blood from the latter, to calf 55. Calves 95, 96 and 106 were inoculated with a pure culture of bovine leptospiras isolated from a human subject. The disease ran the same course in all the animals of this group. Blood urea was high in the terminal stages in all cases. In calves 55, 95 and 96 the jaundice was accompanied by a sudden fall in the erythrocyte count. The animals died between the seventh and the tenth day after inoculation.

Some Details of the Necropsies.—Serous and mucous membranes and adipose tissues were jaundiced in varying degrees. The liver was of normal size and consistency, and its color was normal or slightly yellowish. The cut surface of the liver revealed no gross changes. The spleen was slightly softer than usual. The kidneys were without macroscopic changes except for an icteric tinge and moderate hyperemia of the cortex. The urinary bladder contained dark and reddish brown urine. There were scattered hemorrhagic areas in the lungs. Bacteriologic culture of the organs revealed a paratyphoid-B infection in calves 44 and 55. Cultures of the blood used for further animal passages showed no bacterial growth.

Microscopic Observations.—(*a*) Liver: There was marked hyperemia of the sinusoids and of the portal branches. The structure was well preserved except in scattered areas where the cells were detached from one another; in 1 animal (45) this lesion had spread over extensive areas (fig. 1 *A*). Small groups of liver cells were slightly enlarged and showed discrete vacuolation of the cytoplasm. Fatty change could not be demonstrated with sudan III. Only in 2 calves (95 and 55) was necrosis of liver cells observed. In 1 animal (95) discrete scattered foci of necrosis were present, and these were infiltrated by polymorphonuclear leukocytes. They were seen especially in the vicinity of central veins (fig. 1 *B*). In calf 55, from which paratyphoid bacilli had been isolated, fairly large necrotic nodules were seen. In contrast to the necrotic foci in calf 95, the nodules in calf 55 included cells with large vesicular nuclei and with only a few granulocytes in addition to nuclear debris. When the necrotic nodules were adjacent to larger branches of the hepatic vein, there was evidence of phlebitis and mural thrombosis.

Kupffer cells were generally small, with dark nuclei. Some irregularly distributed Kupffer cells, however, were enlarged and rounded, and exhibited vacuoles and inclusions which resembled erythrocytes in their size and in their manner

of staining with eosin. The prussian blue reaction for hemosiderin was positive in the majority of Kupffer cells. Sparsely distributed, discrete sudanophil granules were demonstrated in them with sudan III. Macrophages containing hemosiderin were seen in the periportal tissue in several animals. In its smaller ramifications the periportal tissue displayed infiltrating cells, mainly accumulated around bile ducts. The latter frequently contained bile thrombi (fig. 1 *D*) which in some instances included desquamated epithelial cells. The accumulations of cells were composed chiefly of lymphocytes, a few plasma cells and histiocytes, and in a

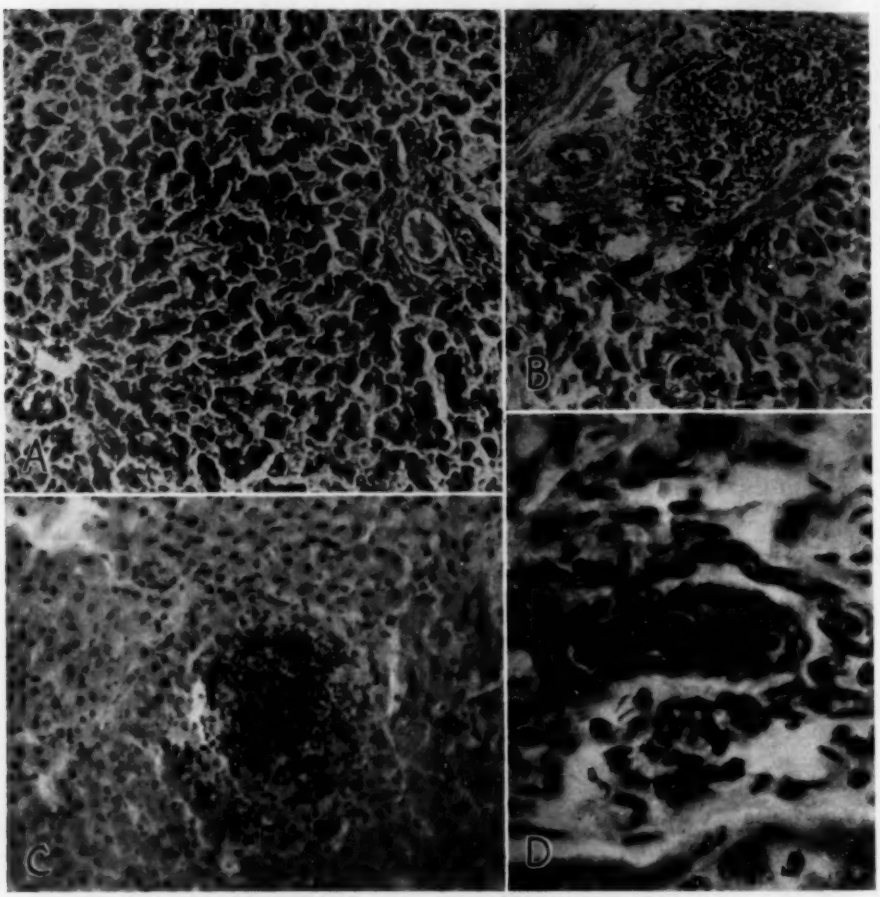

Fig. 1.—*A*, dissociation of liver cords of calf three days after onset of severe jaundice. × 140.

B, cholangitis occurring in the liver shown in A. × 125.

C, necrotic focus infiltrated with polymorphonuclear leukocytes in the liver of a calf dying during an attack of severe jaundice. × 170.

D, cholangitis and bile thrombus in the liver of a jaundiced calf. × 560.

few places some polymorphonuclear leukocytes were also present (fig. 1 *C*). The closeness of the infiltrations to the bile ducts and the observed changes within the latter justify the diagnosis of cholangitis.

The sections treated by Levaditi's method revealed leptospiras in the livers of the jaundiced animals in sparse numbers only. Some organisms were found within liver cells; others were seen among detached cells or lying near the walls of sinusoids (fig. 2 *B*). In the latter instances the characteristic hook of the single, well preserved organism made it possible to distinguish it from the reticular fibers which sometimes became impregnated with silver. In 1 calf (106) clusters of leptospiras were demonstrated in the blood of the larger ramifications of the portal vein (fig. 2 *A*).

(*b*) Spleen: The malpighian bodies were small, without germinating centers. The sinusoids were narrow, and their endothelium appeared normal. The intersinusoidal tissue contained abundant erythrocytes and moderate and approximately equal numbers of polymorphonuclear leukocytes and reticular cells. The prussian blue reaction was positive in small, irregularly distributed groups of cells. In calf 55, which was found to harbor a latent paratyphoid infection, the spleen contained nodular necrotic foci containing nuclear debris.

(*c*) Kidneys: The glomeruli were moderately hyperemic, and single erythrocytes were occasionally present within Bowman's capsule. In many areas the tubules were distended, and albuminous and leukocytic casts occurred, especially in the straight portions. The epithelium exhibited a fair number of necrotic cells in irregular distribution.

In 2 animals (45 and 95) the epithelium had undergone remarkable changes which require more detailed description. In extended portions of convoluted tubules the cells were enlarged, the nuclei being in a basal position, the cytoplasm showed marked vacuolation and contained pigment, which took a reddish tinge like that of hemoglobin when stained with eosin (fig. 2 *C*). In some instances several small coalescing vacuoles contained spherical elements which resembled erythrocytes in size and outline; they stained only faintly in the center but were defined by a bright reddish rim. The prussian blue reaction was strongly positive in many of these vacuolated cells as well as in several casts containing desquamated epithelial cells. The stain for hemoglobin recommended by Dunn and Thompson [8] was tried, but with negative results.

In all animals focal intertubular accumulations of cells were present (plasma cells, large histiocytes, lymphocytes and a few polymorphonuclear leukocytes). The infiltrations were found mainly between convoluted tubules, sometimes approaching the external border of Bowman's capsule. In rare instances, when the infiltrations had broken into tubules the latter contained leukocytic casts.

Silver impregnation revealed moderate numbers of leptospiras in the epithelium of tubules and in the tubular lumens.

GROUP II.—These animals died after a moderately prolonged course of the disease, which was unaccompanied by jaundice (calves 65 and 76).

Summary of Clinical Observations.—Calf 65 was infected with the blood of the jaundiced calf 55, and calf 76, with blood taken from calf 69 (see group III) during the febrile period. Both animals died without development of jaundice twenty-one and sixteen days, respectively, after inoculation. They had high temperatures, leukocytosis, high blood urea and, transiently, traces of bilirubin in the serum. Leptospiras were cultured from the blood of both animals.

Some Details of the Necropsies.—The tissues were not jaundiced. The liver was dark brown and the gallbladder moderately filled. The kidneys were markedly congested, and their cut surfaces were reddish brown. The spleen was without

8. Dunn, R. C., and Thompson, E. C.: Arch. Path. **39**:49, 1945.

gross abnormalities. Subserous petechiae were seen over the entire surface of the stomach, and small hemorrhages were observed in the gastric mucosa.

Bacteriologic examination of the organs revealed paratyphoid-B infections due to serologically distinct strains in both animals.

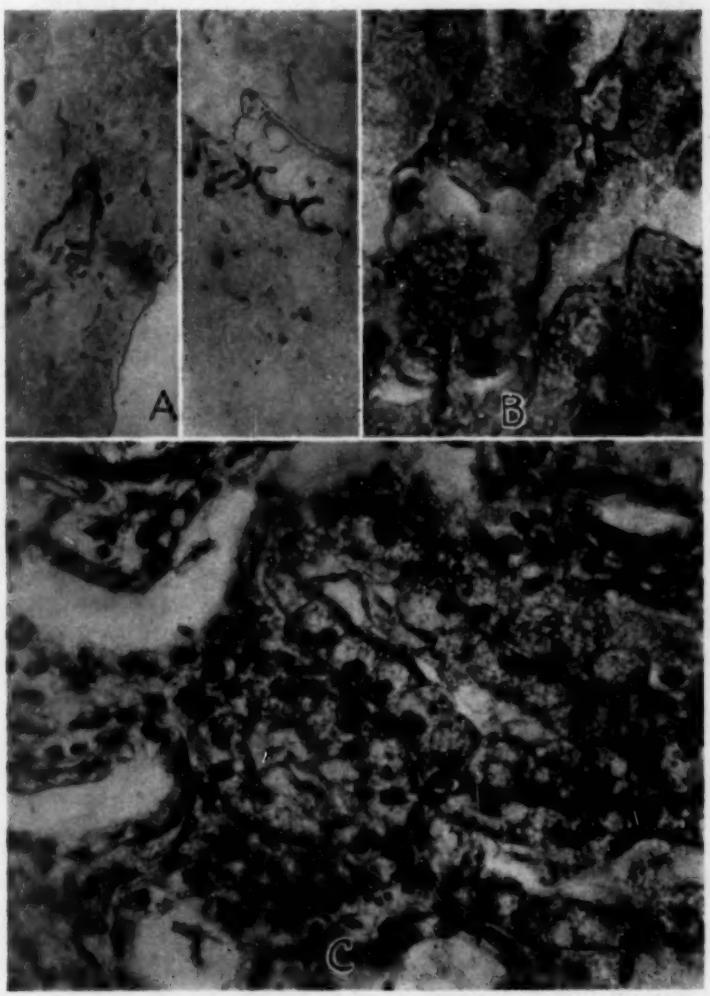

Fig. 2.—*A*, leptospiras lying within the lumen of a portal vein of the liver of a jaundiced calf. × 1,400.

B, organisms lying between liver cells. × 1,400.

C, kidney of a calf with severe jaundice accompanied by hemoglobinuria. The tubular epithelium showed a diffuse reddish tinge like hemoglobin and vacuoles containing droplets staining with eosin as do erythrocytes. × 350.

Microscopic Observations.—(*a*) Liver: The acinous structure and the hepatic cords were left intact except for moderate centroacinic dissociation in calf 76.

There were scattered necrotic foci as previously described in the case of calf 55, which were considered to be so-called "paratyphoid nodules." Moderate hypertrophy of the Kupffer cells was noted. The periportal tissue contained scattered histiocytes which gave a positive prussian blue reaction. Seveal small bile ducts contained bile thrombi, and adjacent to them appeared accumulations of plasma cells, lymphocytes and scattered polymorphonuclear leukocytes such as were observed in jaundiced animals. Levaditi's stain showed single leptospiras within intact liver cells.

(*b*) Spleen: The malpighian corpuscles seemed normal. The endothelium of the sinusoids was flattened, and the lumens were not dilated. Reticular tissue contained numerous reticulum cells, small dark round cells and plasma cells in moderate numbers. The prussian blue reaction revealed abundant macrophages containing hemosiderin granules in calf 65.

In both animals irregularly distributed nodular necrotic foci of the type described as paratyphoid nodules were seen.

(*c*) Kidneys: The glomeruli were without abnormalities. The tubules were normally wide, while in convoluted portions the epithelial cells were swollen, displaying granulated cytoplasm and a moderate loss of nuclei. Now and then the cortex was found to include intertubular accumulations of lymphocytes and a considerable number of plasma cells. In some areas there was evidence that the infiltrating cells had penetrated into tubules which contained leukocytic casts. Levaditi's stain disclosed single leptospiras in the lumens of tubules close to the epithelial border.

GROUP III.—These animals achieved clinical recovery after a mild attack (calves 60, 61, 64 and 69).

Summary of Clinical Findings.—Calves 60, 61 and 64 were infected with blood of the jaundiced calf 55. They reacted with fever, leukocytosis and a slight rise of blood urea. All 3 animals recovered. They were killed outside the laboratory one week after the fever had subsided, i. e., twenty-one days after inoculation.

The fourth animal of this group (69) was infected with blood of calf 65 (see group II). It reacted, as did the other 3 animals, with fever and leukocytosis for several days, and there were transient traces of bilirubin in the serum. The blood urea rose to 129 mg. per hundred cubic centimeters on the twelfth day after inoculation. This animal was killed for examination while in a state of perfect clinical health fifty-nine days after inoculation.

Some Details of the Necropsies.—All the animals were free of macroscopic changes except calf 69, which exhibited severe renal damage. The kidneys of this animal were considerably enlarged, weighing 550 Gm. each. They were of doughy consistency, and their cut surfaces were moist and pale reddish gray. No abnormalities were found in other organs. No bacterial infection was observed in the blood and organs of these animals.

Microscopic Observations.—(*a*) Liver: There were identical findings in all the 4 animals of this group. The acini were well formed, but the trabeculae frequently were irregular in outline because of cellular hyperplasia (fig. 3*A*). Multinuclear cells were rare, and mitotic figures were not found.

The periportal tissue was sparsely developed and poor in cells. Kupffer cells were flattened and had dark nuclei. Hemosiderin stains and fat stains gave negative results. No leptospiras were found.

(*b*) Spleen: The malpighian corpuscles showed no abnormalities. There was pericorpuscular hyperemia, accompanied by accumulations of polymorphonuclear

leukocytes. The sinusoids were widely open and lined with small dark endothelial cells. In 1 animal the sinusoids were small and seemed more numerous than usual, suggesting the presence of so-called sinus hyperplasia. The reticular tissue was sparsely developed and contained scattered plasma cells. The prussian blue reaction was negative.

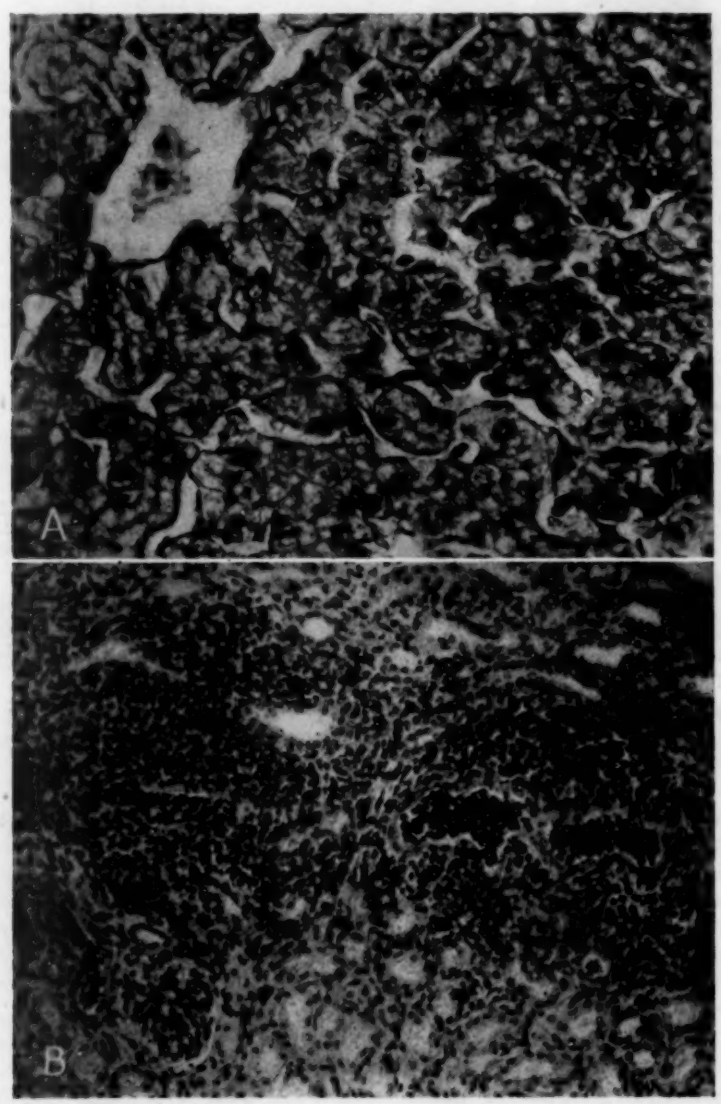

Fig. 3.—*A*, liver of a calf which was not jaundiced, from group III. Note the hypertrophy of the liver cords, which may be attributed to hyperplasia of liver cells. Foot's silver impregnation; × 450.

B, kidney of a calf 59 days after inoculation. This animal recovered completely after slight illness. Note severe interstitial nephritis breaking into the tubules. × 160.

(*c*) Kidneys: The kidneys of calves 60, 61 and 64 revealed no changes except for occasional loss of nuclei of the epithelium of the convoluted tubules. Leptospiras could not be demonstrated.

In the kidney of calf 69 microscopic lesions were observed as follows:

The glomeruli were without abnormalities. In all parts of the kidney there was an abundant interstitial cellular infiltrate, predominantly composed of plasma cells and lymphocytes. The central portions of some foci of infiltration contained accumulations of polymorphonuclear leukocytes. In several areas, mainly in the pyramidal portions, small abscesses were present. The tubules adjacent to cellular infiltrations frequently contained leukocytic casts (fig. 3 *B*). Silver impregnation revealed abundant leptospiras in the epithelium and in the lumens of convoluted tubules in areas which were free of inflammatory exudate. Many fragmented

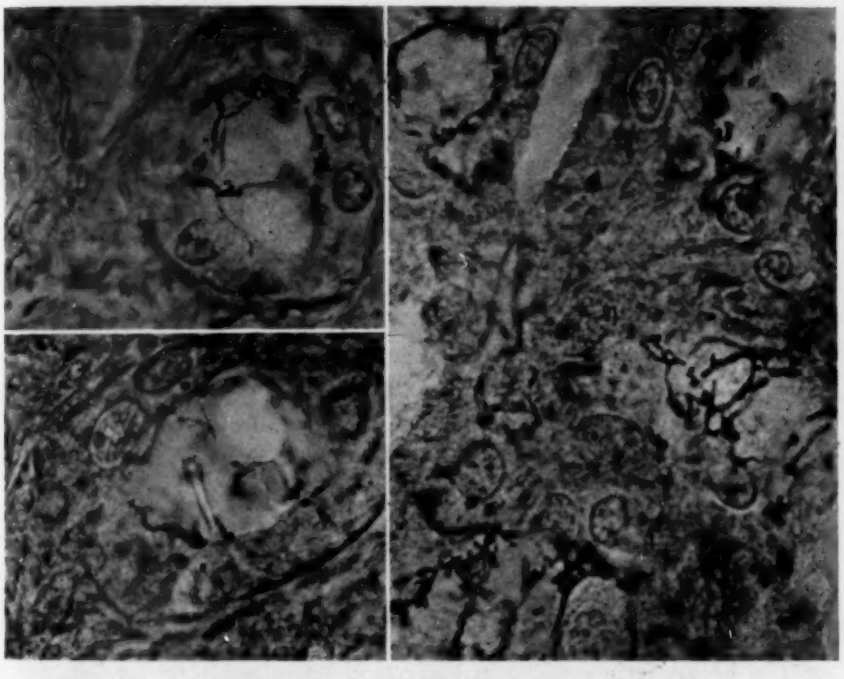

Fig. 4.—Leptospiras in renal tubules of the kidney shown in figure 3 *B*. × 1,400.

leptospiras were present, together with typical complete organisms. In some places the entire lumen of a tubule was lined with a thick network of leptospiras (fig. 4).

COMMENT

Nearly all previous reports on the pathologic aspects of bovine leptospirosis have dealt with observations in field cases. It seems, therefore, worth while to compare these observations with ours, made on experimentally infected animals.

Changes in the Liver.—In the livers of 7 of 8 calves which succumbed to leptospirosis in its fulminant or in its protracted form, dissociation

of hepatic cells was noted, while necrotic foci were found only three times. This experience is not shared by other authors. Dissociation was also recorded by Jungherr,[3] but the low incidence of regressive changes of liver cells in our material does not agree with the experience of others,[9] who reported the frequent occurrence of necrotic foci. Moreover, such foci were seen by us, with only a single exception, in animals harboring paratyphoid bacilli and were probably caused by this concurrent infection. This view is supported by the histologic characteristics of the necrotic foci, which are identical with the well known paratyphoid nodules,[10] by their simultaneous presence in the spleen and by their occurring whether the animals were jaundiced or not.

There remains only a single case in which necrotic foci were found in the liver of an animal which died at the height of a rapidly fatal attack of jaundice and was found free of concurrent infection. These foci were different from the paratyphoid nodules by their centroacinic location, their small size and the presence of polymorphonuclear leukocytes.

In our experience the periportal tissues seemed to be more constantly injured than the hepatic epithelium. In these tissues, infiltrations of small round cells were found, predominantly adjacent to the bile ducts, which frequently contained bile plugs and desquamated epithelium, and the diagnosis of cholangitis thus seemed justified. Periportal infiltrations were also mentioned by Avrorow. A possible explanation of this observation may be found in the occurrence of abundant leptospiras in the portal vein, as seen in an animal of our series. It is conceivable that the low pressure of the portal circulation allows the blood-borne leptospiras to be in prolonged and intimate contact with the periportal tissues.

Parenchymal retention of bile was found in the liver of only a single animal, which had necrotic foci in the liver in the absence of paratyphoid infection. The retention of bile seemed therefore to play only a minor role in the production of jaundice.

The hemosiderin present in the reticuloendothelial cells and in the liver cells of jaundiced animals points to the important role played by the destruction of red blood cells in the origin of jaundice. Further evidence of this destruction lies in the fact that in 3 of 6 jaundiced animals the erythrocytes of the peripheral blood decreased in number suddenly at the time of the appearance of the jaundice.

Changes in the Spleen.—The lesions of the spleen were negligible and not specific in nature. In the grave icteric form as well as in the protracted nonicteric form of the disease congestion and hemosiderosis

9. Mathews.[8] Avrorow.[6]

10. Joest, E.: Leber und Gallenwege, in Frei, W., and others: Joest's Handbuch der speziellen pathologischen Anatomie der Haustiere, ed. 2, Berlin, Verlagsbuchhandlung von Richard Schoetz, 1936, vol. 2, pt. 1.

were found. There were no abnormalities in animals which recovered following a mild illness. In no instance could leptospiras be demonstrated.

Our own experience corresponds to that of other authors, except Mathews, who found atrophy of the lymphoid tissue and persistent hemosiderosis in cases with infection lasting two to three weeks. Our, on the whole, negative findings agree also with reports on canine leptospirosis and on human cases of Weil's disease.

Changes in the Kidneys.—In the acute icteric form a moderate degree of degeneration of the tubular epithelium was present. In several jaundiced animals a peculiar vacuolar change of the epithelium was seen, accompanied by different kinds of pigmentation, which had also been observed and described by Mathews. As in his case, we observed pigmentation giving a positive iron reaction in certain portions of the tubules. In others, the epithelial cells contained pigmented iron-negative vacuoles which stained with eosin precisely as did erythrocytes but failed to stain specifically with the Dunn-Thompson method recommended for the demonstration of hemoglobin pigment.

In addition to the regressive lesions, interstitial infiltrations were found in the kidney as early as seven days and as late as fifty-nine days after inoculation. The infiltrations appeared in both jaundiced and nonjaundiced animals. They were present only in kidneys containing leptospiras. The organisms were not found within the areas of infiltration but only within renal tubules with well preserved epithelium.

Interstitial nephritis was observed in leptospirosis of cattle by Mathews. He observed this lesion only when the clinical manifestations of the illness had lasted longer than two to three weeks. The question arose whether the interstitial nephritis which appeared as early as the first few days of illness was really due to the presence of leptospiras or to injury not related to these organisms.

In particular it was necessary to exclude the possibility of interstitial nephritis, which is endemic among young calves in some countries. This condition is known as "white-spotted kidney."[11] We felt that the presence of this disease could be excluded for various reasons: The kidneys of our experimental calves never showed the "whitish patchy discoloration" of the surface described by Theobald Smith. This author considered the disease to be probably due to Bacillus coli infection of newborn calves, but that organism was never found at autopsy in our cases. Furthermore, the endemic form of interstitial nephritis is said to heal spontaneously with residual scars during the second month of life, whereas in our cases infiltrations were present without evidence of fibrosis up to the end of the third month.

11. Smith, T.: J. Exper. Med. **41**:413, 1925. Nieberle, K.: Interstitielle Nephritis, in Nieberle, K., and Cohrs, P.: Lehrbuch der speziellen pathologischen Anatomie der Haustiere, Jena, Gustav Fischer, 1931.

The lesions observed in calves when disease is acute are essentially identical with those seen in human patients infected with leptospiras from bovine leptospirosis [12] and in Weil's disease.[13] In regard to the human infection, the absence of degenerative lesions of the liver and the small round cell infiltration of the periportal tissue are stressed by most authors.

Interstitial nephritis as seen in our animals has long been known to occur in patients with Weil's disease, even in nonjaundiced ones.[14] The major importance of renal as compared with hepatic lesions has again been emphasized recently with regard to the experimental infection of guinea pigs with Leptospira hemorrhagica.[15]

In the bovine as in the human disease there is no apparent correlation between the intensity of the structural lesions and the clinical manifestations of the disease; the lesions found in our material were almost identical in the jaundiced and in the nonjaundiced calves except that varying degrees of hemosiderosis were observed in the jaundiced group. The diseases in man and cattle also have in common the fact that leptospiras occur in the liver and in the kidneys independently of cellular infiltrations. Further, it is found in both the human and the bovine material that the leptospiras persist in the kidneys for several weeks in cases distinguished by a mild clinical course.

SUMMARY

Young calves experimentally infected with a bovine strain of leptospiras were examined at necropsy. A description is given of the structural changes observed in the liver, the spleen and the kidneys in three different clinical forms of the disease (an acute form accompanied by jaundice, a subchronic form without jaundice and a form with a mild course leading to clinical recovery).

Leptospiras were demonstrated by the Levaditi method in the liver and the kidneys of every animal in the jaundiced and the subchronic, nonjaundiced group. They were also present in a kidney of 1 calf two months after inoculation. This animal suffered only a slight attack and appeared to recover completely.

The histologic lesions observed were essentially similar to those reported in human Weil's disease.

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12. Caspar, J., and Reif, L.: Harefuah **30**:113, 1946.

13. Jeghers, H. J.; Houghton, J. D., and Foley, J. A.: Arch. Path. **20**:447, 1935. Ashe, W. F.; Pratt-Thomas, H. R., and Kumpe, C. W.: Medicine **20**:145, 1941.

14. Stiles, W.; Goldstein, J. D., and McCann, W. S.: J. A. M. A. **131**:1271. 1946.

15. Wylie, J. A. H.: J. Path. & Bact. **58**:351, 1946.

MEDULLARY HYPERPLASIA OF THE ADRENAL GLAND IN AGED WISTAR ALBINO AND GRAY NORWAY RATS

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THIS paper presents a description of medullary hyperplasia of the adrenal gland observed in old animals that were studied in a cooperative investigation of changes occurring with age in the tissues and organs of rats. Findings based on the weights of the adrenal glands, the relative volumes of cortex and medulla and the histologic alterations of the cortex will be reported separately.[1]

MATERIALS AND METHODS

Rats of the Wistar albino and the gray Norway strain, born and raised in the Wistar colony, were used. In this study the old animals ranged from 700 to over 1,000 days of age, and included 16 female and 15 male albino and 3 male and 3 female gray Norway rats.

The adrenal glands were dissected free of connective tissue, and, except in 1 instance, the two glands were weighed together. One of the adrenal glands from each animal was fixed in Bouin's fluid, sectioned serially and stained with hematoxylin and eosin. The other gland was fixed in Zenker's fluid, and sample sections were stained either with hematoxylin and eosin or with Masson's or Mallory's triple stain.[2] With the exception of 3 of the gray rats, microscopic sections were made of both adrenal glands.

OBSERVATIONS

Although the adrenal glands of younger rats were also examined, medullary hyperplastic changes were seen only in the glands of the old animals, 700 days or more of age. They were found in the glands of 13 of the male and 3 of the female rats. The weights of the adrenal glands of these animals, with estimations of the degrees of hyperplasia, are listed in the table. The weights of the left and the right gland separately were known for 1 rat only (2s ♂). However, it may be seen that in many instances the growths were sufficiently large to increase the weights of the glands materially.

Hyperplasia of the medulla was either nodular or diffuse, and ranged in extent from small nests of cells (+) to complete, or almost complete,

From the Wistar Institute of Anatomy and Biology.

This investigation was aided by a grant from the Samuel S. Fels Fund.

This work is part of a general study of aging organized by Dr. E. J. Farris, of the Wistar Institute, and carried on by cooperating scientists.

1. Yeakel, E. H.: To be published.

2. Most of the sectioned material was prepared by Dr. Eugene Cutuly, who withdrew from the program for the study of aging to enter the armed forces.

involvement of the medulla (+ + +). Only minimal hyperplasia was found in the adrenal glands of the gray rats.

Minimal hyperplasia occurred as more or less well defined nodules within the medullary tissue, distinguished only by cytoplasm that stained more deeply than that of the surrounding cells. Similar but larger nodules tended to protrude into the cortex and to distort the normal contour of the medulla. Slight dilatation of the sinusoids of the nodule was usually seen. Larger growths took the form of nodular hyperplasia surrounding an enlarged vascular channel the walls of which were frequently thickened. The tissue of the circumscribed nodule was hyperchromatic, and was either compactly arranged or separated into distinct strands and nests of cells by dilated sinusoids. The largest growths consisted, again, of a relatively solid mass of tissue or of one broken up

The Weights of the Adrenal Gland That Showed Hyperplasia of the Medulla (Sum of Weights Both Glands)

Rat	Sex	Age, Days	Adrenal Weight, Gm.	Abnormal Glands	Degree of Hyperplasia *
410	♂	903	0.1918	One	+++
398	♂	946	0.0790	One	+++
473	♂	1,057	0.1651	Both	+++
2 S	♂	1,024	0.1396	One	+++
			0.0606	One	++
60	♂	949	0.0725	One	++
127	♀	903	0.0867	Both	++
830	♂	700	0.0485	Both	++
4 S	♂	1,006	0.0834	Both	++
999	♂	1,011	0.0883	One	+(+)
387	♀	901	0.0860	One	+
808	♂	700	0.0885	One	(+)
90	♂	900	0.0440	Both	(+)
954	♂	900	0.0473	Both	(+)
5 †	♀	1,112	0.0681	One ‡	+
17 †	♂	1,057	0.0810	Both	+
60 †	♂	1,055	0.1114	One ‡	(+)

* The degree of hyperplasia ranged from presence of small nests of cells (+) to complete or almost complete involvement of the medulla (+++).
† The rat was of the gray Norway strain.
‡ Only one gland was examined.

into cords of cells by sinusoidal dilatation. The mass encroached on the cortex and compressed or eliminated it. In 2 instances invasion of the capsule was observed.

The following descriptions of the gross and the microscopic appearance of individual glands are presented in a descending order of degree of hyperplasia.

410 ♂. The right adrenal gland of this rat appeared to be normal grossly and microscopically. The left on dissection was enlarged, discolored and hemorrhagic, with a cream-colored lump at the upper pole. The left adrenal vein was wide and tortuous. Microscopic sections of this gland revealed that the cortex was almost completely eliminated, the bulk of the gland being made up of tissue that resembled normal medulla but contained enlarged sinusoids (fig. 1). Some of this tissue lay outside the capsule, comprising the cream-colored protrusion noted on dissection. The cells of the growth contained clear vesicular nuclei and were uniformly stained. The sinusoids were lined with a delicate endothelium.

398 ♂ The right adrenal gland was small and of normal microscopic appearance. The left gland was enlarged, and the medulla resembled that of 410 ♂, just described.

473 ♂. Grossly, both adrenal glands were discolored and irregularly shaped, with a long protrusion at one pole of the left gland. Microscopic sections (fig. 2) showed a greater amount of cortical tissue than was observed in the adrenal gland of 410 ♂, but the same type of disorganization of the medullary tissue. However, unlike the first 2 glands described, much of the lining of the sinusoids was hypertrophic, and several areas of fibrosis were present in the medulla. The protrusion of the left adrenal gland was composed of less loosely arranged medullary tissue.

In contrast to the glands already described, the right adrenal gland of this rat (fig. 3) contained a large, bilobed medulla made up of solid tissue, with few vascular spaces. Occasional heavy strands of connective tissue were seen. Cells located on the periphery of both lobes were stained yellow (Zenker fixation, Mallory stain), but those in the interior were pink.

2 S ♂. Except for a narrow rim of cortical tissue a few cells in depth, the entire right gland was made up of hyperplastic medulla. The cells were solidly packed, and those at the periphery of the growth were yellow (Zenker, Mallory) as in the right adrenal gland of 473 ♂. Blood channels with thickened lining were present in the interior but were not especially prominent.

In the glands just described, no normal medullary tissue was found. The remainder of the adrenal glands contained varying amounts of normal and hyperplastic medulla.

60 ♂. Microscopically, one adrenal gland of this rat was normal, but the bulk of the medulla of the other gland was composed of tissue that was separated into distinct cords by large vascular spaces, which were lined with heavy strands of tissue stained blue with Mallory's triple dyes (fig. 4). Grossly, the gland had appeared to be normal. In the hyperplastic region the cytoplasm of the cells was deeply stained, most heavily in the center of the mass. Adjacent to this area there was a triangular cap of medullary tissue that was normal (fig. 5). The cells here were compactly arranged, their cytoplasm was faintly stained and granular, and the nuclei were smaller and more chromatic than those seen in the hyperplastic area.

127 ♀. In both adrenal glands a portion of the medulla was of normal appearance and a part was similar to the hyperplastic medulla of 60 ♂.

830 ♂. The adrenal glands appeared to be normal grossly. Microscopically each gland showed a well circumscribed area of more heavily stained cells lying eccentrically in the normal medullary tissue. In one gland the nodule surrounded a dilated blood vessel, the walls of which were thickened (fig. 6). The cells were closely packed. In the other gland the cells were loosely arranged in cords and nests; capillary endothelium was normal in appearance.

4 S ♂. Within the normal medulla of the right gland there was a large eccentric mass of fairly solid tissue surrounding a large blood sinusoid, the lining of which was thickened. With Mallory's stain, the outer cells of the mass were yellow. There were also patches of hyperchromatic cells, like those of the nodule, within the normal medullary tissue.

The medulla of the left gland of this rat was enlarged. In one area the tissue was hyperplastic and solidly arranged, and bulged into the cortex; in another region normal medullary tissue was seen. Enlarged vascular channels were present in

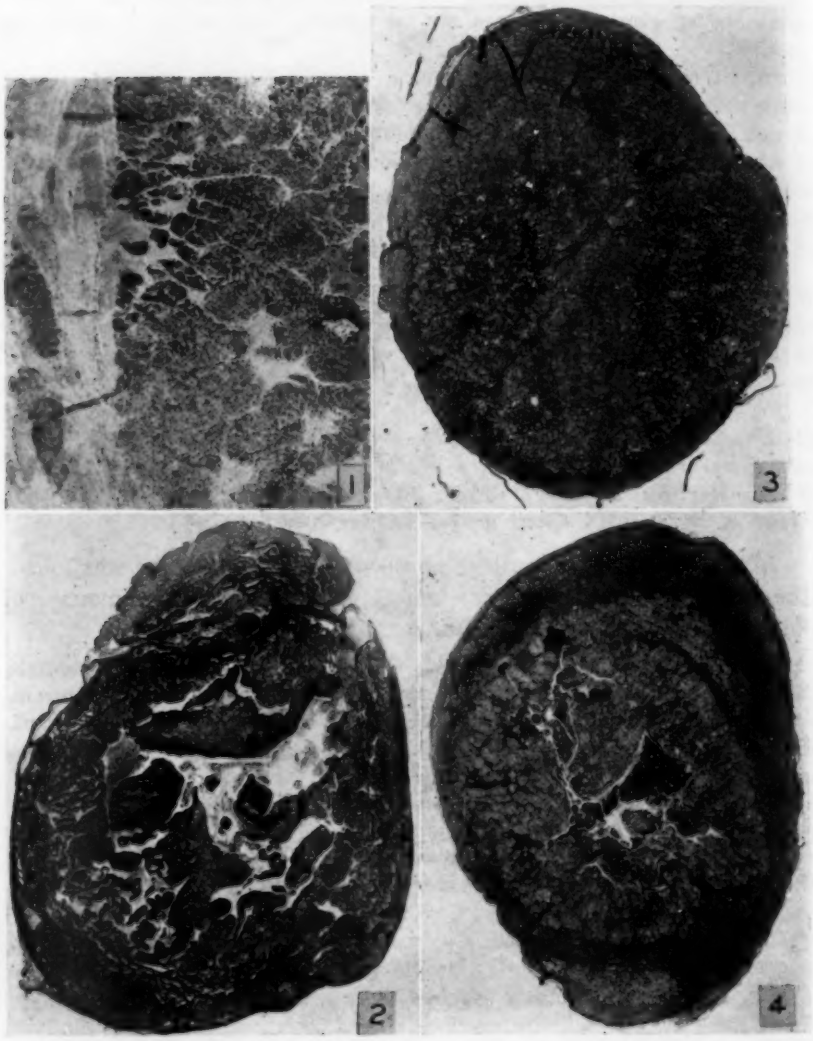

Fig. 1. (410 ♂).—Hyperplastic medullary tissue. On the right it extends to the capsule; on the left it is outside the capsule. Bouin fixation; hematoxylin and eosin stain; × 51.5.

Fig. 2 (473 ♂).—Cortex reduced to a narrow rim. At the top the medullary tissue is outside the capsule. There are large vascular spaces and heavily stained areas of connective tissue within the hyperplastic medulla. Bouin fixation; hematoxylin and eosin stain; × 15.5.

Fig. 3 (473 ♂).—Bilobed hyperplastic medulla with little vascularization. Zenker fixation; Mallory triple stain; × 15.5.

Fig. 4 (60 ♂).—Enlarged medulla with prominent vascular spaces. Note the small portion of normal medullary tissue at the top. Zenker fixation; Masson triple stain; × 15.5.

the interior of the medulla. Their endothelial lining was greatly thickened in places, and two endothelial pearls were noted in the hypertrophic tissue. The medullary tissue about the blood channels was stained more deeply than that of the normal region and appeared to be hyperplastic—resembling, on a small scale, the adrenal

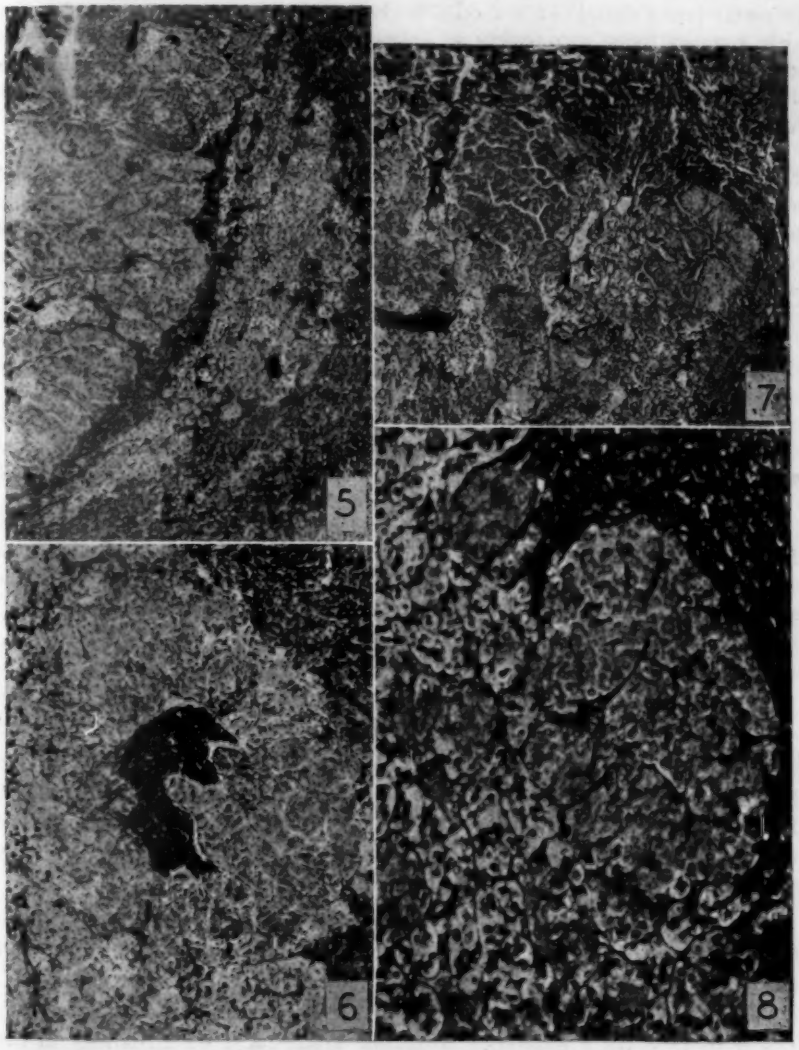

Fig. 5.—Detail of figure 4 showing (from left to right) hyperplastic medulla, normal medulla and cortex. × 71.

Fig. 6 (830 ♂).—Nodule surrounding a blood vessel. Normal medulla is seen at the bottom, to the left; cortex, at the right. Zenker fixation; hematoxylin and eosin stain; × 53.

Fig. 7 (387 ♀).—Two hyperplastic areas protruding from the medulla at the left. Bouin fixation; hematoxylin and eosin stain; × 53.

Fig. 8 (17 G ♂).—High magnification of a small nodule. Normal medullary tissue is seen on the left; cortex (dark stain), on the right. Zenker fixation; Mallory triple stain; × 141.5.

gland of 473 ♂ page 73). There was a gradual transition between the normal region, the solid hyperplastic area and the loosely arranged tissue.

2 S ♂. The right adrenal gland of this rat was described on page 5. The medulla of the left gland was enlarged and distorted in shape. Part of it was composed of a solid mass which extended to the edge of the gland and compressed the cortex into a strand of a few cells' thickness. Another portion was made up of fairly compact tissue surrounding an enlarged vascular channel. The lining was greatly thickened, and occasional epithelial pearls were seen in it. A third region of the medulla appeared to be normal histologically, with cytoplasm that took a lighter stain than the other two areas.

999 ♂. The adrenal glands were thought to be normal on dissection. Microscopic sections of the medulla of one gland showed a fairly well circumscribed nodule surrounding a blood vessel. The sinusoids of the nodule were slightly dilated. The cells resembled those of the normal portion of the medulla except for the fact that the cytoplasm of the former (with Mallory's triple stain) was deep pink, while the normal cells were faint salmon or yellow. Patches of similar hyperchromatic cells were scattered throughout the medulla.

387 ♀. In the medulla of one adrenal gland were nodules and groups of cells with homogeneously stained cytoplasm that took the dye more readily than the surrounding tissue. They resembled the patches described in the medulla of 999 ♂; but some projected into the cortex, giving the medulla an irregular outline (fig. 7). Slight but definite dilatation of the sinusoids was present. Elsewhere in the serial sections of this gland several sinusoids were found to be greatly enlarged and filled with erythrocytes. The endothelium was normal in appearance.

Similar aggregates of prominent hyperchromatic cells were found in the medullas of 828 ♂, 90 ♂ and 954 ♂. In the adrenal glands of the 6 gray Norway rats that were examined, only minimal hyperplastic areas of this type were seen. A nodule in the adrenal gland of a 1,000 day old male gray is shown in figure 8.

COMMENT

The degrees of hyperplasia described suggest the possibility that they may represent stages in the development of a neoplasm. Ewing stated that "in many situations adenomatoid hyperplasia becomes progressive and passes into a neoplastic process," [3a] and referring to struma adrenalis, affecting medulla as well as cortex, he remarked that "between the nodular hypertrophies and true adenomas there appears to be every transition." [3b]

In glands in which the medullary hyperplasia had advanced beyond a minimal stage it was associated with an unusual dilatation of one or more sinusoids. Ewing wrote that while "some adenomas . . . represent chiefly an excessive response to functional stimulus, others appear to owe their existence to minor anatomical disturbances in the blood supply." [3a]

Probably identical changes were described by Staemmler [4] as adenoma of the adrenal medulla of rats with chronic nicotine poisoning. He

3. Ewing, J.: (*a*) Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1942, p. 508; (*b*) p. 512.

4. Staemmler, M.: Virchows Arch. f. path. Anat. **295**:366, 1935.

attributed the appearance of the growths to the repeated stimulation of the medullas by the daily subcutaneous injection of nicotine. In a paper dealing with medullary hypertrophy following suppression of thyroid secretion induced with thiouracil, Marine and Baumann [5] reported an enlarged medulla observed in a female rat over a year old, the description of which resembles the extensive hyperplasia seen in 410 ♂ and 398 ♂ (page 73).

No experimental procedures were carried out on the rats used in this study of aging. Nevertheless, the work of Staemmler [4] and of Marine and Baumann [5] suggests that the functional state of the other endocrine glands may play a part in the development of the medullary growths and that experimental alterations of hormonal balance may simulate changes that occur spontaneously with advancing age.

Findings reported here point to a sex difference in the frequency of the medullary hyperplasia. Of the 36 glands from 19 old female rats of both strains that were examined, 4 (11 per cent) showed some degree of medullary growth; these changes were found in 3 rats (16 per cent). Eighteen of the 35 glands from old males exhibited hyperplasia, including all of the most advanced stages. The growths were found in 13 of the 18 males (72 per cent); only 5 rats possessed adrenal glands with no morphologic abnormalities of the medullary tissue.

SUMMARY

Adrenal glands of 16 female and 15 male albino rats and of 3 male and 3 female gray Norway rats, 700 days or older, were examined microscopically. Both glands of the albino rats were studied, but only 9 from the gray rats. Hyperplasia of the medulla was observed in 13 of the male rats (72 per cent) and in 3 of the female rats (16 per cent). Of 36 glands from females, hyperplastic changes were found in 4 (11 per cent); of 35 glands from male rats, the changes were seen in 18 (51 per cent).

Varying degrees of hyperplasia were seen, and when arranged in order of size they suggested the development of an adenoma. The minimal stage consisted of a knot of hyperchromatic cells within the normal medulla. Sinusoidal dilatation was usually present, separating the strands of the medullary cords of the nodule. Similar but larger nodules surrounded enlarged blood channels. More advanced growths were made up either of relatively solid hyperplastic tissue or of tissue separated into cords by greatly dilated sinusoids or of both. In the most advanced stages the cortex was compressed to a narrow rim or was completely obliterated, with no normal medullary tissue being identified. In 2 instances invasion of the capsule was observed.

5. Marine, D., and Baumann, E. J.: Am. J. Physiol. **144**:69, 1945.

Case Reports

TUMOR OF CAROTID BODY TYPE ARISING IN THE MIDDLE EAR

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BOSTON

IN 1945 Rosenwasser [1] described a tumor of the middle ear and the mastoid process having the histologic characteristics of a tumor of the carotid body and suggested that it might have arisen from the glomus jugularis described by Guild.[2]

The following case is reported because of the resemblance of the tumor to that described by Rosenwasser and because it seems probable that other similar tumors are described in the literature under different names.

REPORT OF A CASE

M. P., a 43 year old housewife, had had trouble with her left ear for ten years. Three years before admission she had a "polyp" removed. When examined at the Lahey Clinic on March 20, 1946, she had a polypoid mass of considerable size in the left aural canal. Roentgen examination of the petrous ridges and the mastoid processes showed the right side to be normal, while on the left there were sclerosis and thickening of the septums of the air cells, consistent with chronic mastoiditis. The laboratory findings were not remarkable.

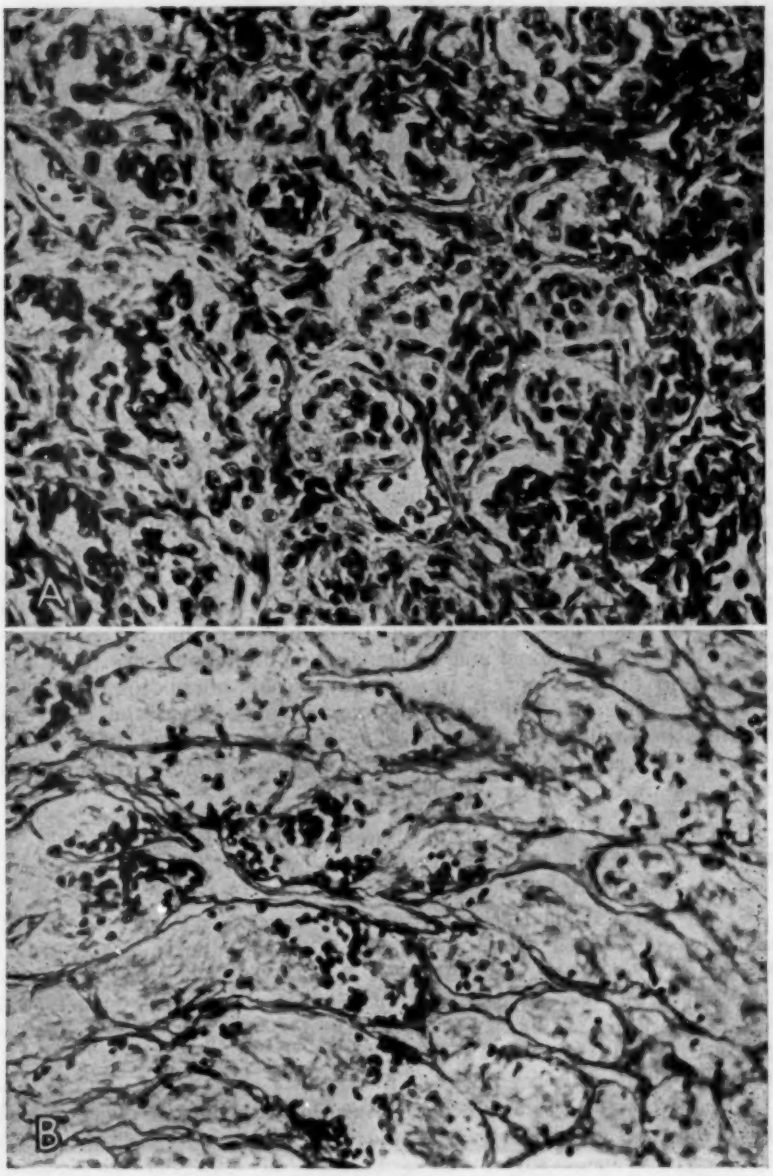
Operation.—On June 15 endaural radical mastoidectomy was done on the left side by one of us (F. D. L.). The antrum was small and filled with some debris. When the middle ear was opened, considerable cellular material somewhat resembling a polyp and suggesting tumor tissue was seen in the region of the orifice of the eustachian tube just anterior to the promontory, eroding the intratympanic portion of the fallopian canal. Profuse bleeding was encountered, apparently arterial, and the patient lost about 1,000 cc. of blood before it could be controlled. She had mild postoperative facial paralysis, but made a good recovery. There was no sign of recurrence one year after operation.

Pathologic Observations.—The material received consisted of about 0.5 cc. of soft grayish red tissue. Microscopically, this material consisted in part of fairly dense fibrous tissue and in part of tumor tissue composed of nests of epithelioid cells surrounded by a fairly delicate fibrous and vascular stroma—a histologic picture typical of carotid body tumor (*A* of figure). The fundamental and characteristic pattern was clearly brought out by silver impregnation (*B* of figure). There was only slight variation in nuclear size in the epithelioid cells, and no mitotic figures were observed.

Both the tumor reported by Rosenwasser and the one described here appeared to have the characteristic structure of tumors arising

From the Laboratory of Pathology of the New England Deaconess Hospital and the Lahey Clinic.

1. Rosenwasser, H.: Arch. Otolaryng. **41**:64, 1945.
2. Guild, S. R.: Anat. Rec. (supp. 2) **79**:28, 1941.



Sections of a tumor of the carotid body type that arose in the middle ear: *A*, eosin–methylene blue stain; × 300. *B*, Wilder silver impregnation; × 300.

in the carotid body as described elsewhere.[3] In neither of these 2 cases was there any evidence of a tumor in the region of the carotid body. Therefore, the suggestion of Rosenwasser that such a tumor might arise in the histologically similar glomus jugularis of Guild[2] seems to be a sound one. The latter structure is said to be situated either in the adventitia of the jugular bulb, immediately below the floor of the tympanic cavity, or in the canal that transmits the ramus tympanicus of the glossopharyngeal nerve. Guild stated that the innervation and the blood supply of this body come from the same sources that supply the carotid body, namely, the glossopharyngeal nerve and the ascending pharyngeal artery. The suggestion is thus a reasonable one, and if future investigations disclose that the glomus jugularis functions as a chemoreceptor, the analogy could be considered complete.

The literature apparently contains no reports of other cases of tumors of the middle ear resembling tumor of the carotid body. However, the report of Capps[4] of 2 middle ear tumors diagnosed as hemangioendothelioma is accompanied by photomicrographs which suggest that these tumors may have been of the same type as that reported here. (Carotid body tumors, may, of course, resemble endothelioma or "perithelioma" and are so classified by some writers.) It is interesting that in all 4 cases, i.e., those of Capps, that of Rosenwasser and the one reported here, symptoms (usually deafness) had been present for at least ten years. Capps described the tumor as arising from the inner tympanic wall in his case; Rosenwasser, from the hypotympanum.

Several other cases of tumor of the middle ear reported as instances of endothelioma, hemangioma or hemangioendothelioma are to be found recorded in the literature. In some of these[5] no photographs of the tumor are given, and it is impossible to judge from the description whether the structure might have resembled a carotid body tumor.[6] In a few others[7] the illustrated histologic aspect of the tumor is distinctly not that of the carotid body.

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3. LeCompte, P. M.: Am. J. Path., to be published. Bloom, F.: Arch. Path. **36**:1, 1943. Gratiot, J. H.: Internat. Abstr. Surg. **77**:177, 1943.

4. Capps, F. C. W.: J. Laryng. & Otol. **59**:342, 1944.

5. (*a*) Marx, H., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 12, pp. 490-568. (*b*) Figi, F. A., and Hempstead, B. E.: Arch. Otolaryng. **37**:149, 1943. (*c*) Schall, L. A.: ibid. **22**:548, 1935. (*d*) Scott, S.: J. Laryng. & Otol. **54**:601, 1939. (*e*) Beck, J. C.: Illinois M. J. **9**:137, 1906. (*f*) Bryant, W. S.: Ann Otol., Rhin. & Laryng. **16**:301, 1907. (*g*) Calabresi, A.: Osp. maggiore **7**:17, 1919. (*h*) Urbantschitsch, E.: Ztschr. f. Ohrenh. **67**:365, 1913.

6. Dr. F. A. Figi stated (personal communication) that a review of the 7 tumors reported by him and Hempstead[5b] as hemangioendothelioma does not permit reclassification of any of them as of carotid body type. Through the courtesy of Dr. L. A. Schall, we have been able to examine slides of the two tumors reported by him[5c] as hemangioendothelioma; they do not have the structure of carotid body tumors.

7. Sullivan, J. A.: Arch. Otolaryng. **20**:61, 1934. Fischer, J.: Ztschr. f. Hals-, Nasen- u. Ohrenh. **5**:221, 1923. Bronzini, A.: Arch. ital. di otol. **40**:590, 1929. Jones, J. A.: J. Laryng. & Otol. **45**:265, 1930. Specht and Völker: Arch. f. Ohren-, Nasen- u. Kehlkopfh. **120**:93, 1929.

In a discussion of granular cell myoblastoma, Horn and Stout[8] reported two middle ear tumors which they described as having an "organoid" structure. Their figure 2 bears a strong resemblance to a carotid body tumor, and the question may be raised as to whether the tumor illustrated could not be so classified.

Probably most of the polypoid tissue masses removed from middle ears and mastoid processes are not examined histologically. If this were done more often, it seems likely that a small percentage would fall into the category here described.

SUMMARY

A case of tumor of carotid body type apparently arising in the middle ear is described. A possible origin from the glomus jugularis of Guild as proposed by Rosenwasser is considered. It is suggested that other tumors of this type may be recorded in the literature under different names, such as "hemangioendothelioma" and "myoblastoma."

8. Horn, R. C., Jr., and Stout, A. P.: Surg., Gynec. & Obst. **76**:315, 1943.

ARTERIOSCLEROSIS IN INFANCY

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Medical Corps, Army of the United States

THERE have been numerous reports on juvenile arteriosclerosis. Generally, this means arteriosclerosis of persons below the age of 20 years. The number of cases in which the patient was less than 1 year of age is small. Zeek [1] lists 5 such cases up to 1930. Scheidegger [2] reported a case in which the patient was a 7 month old infant. Brown and Richter [3] listed 4 additional cases from the literature and added 1 case of their own. Stryker [4] classified coronary occlusion of infants and children under medial calcification with fibroblastic proliferation of the intima. He recorded 15 cases, 7 of which had not been previously included in a review, and added 4 of his own. Since this condition is apparently rare, the report of an additional case seems worth while.

REPORT OF A CASE

A boy was born Sept. 10, 1946, at full term, after an uneventful labor; he weighed a little more than 7 pounds (3,175 Gm.), had a good color and a good cry and showed no abnormalities on the usual examination. The mother was 21 years old and in good health. She had had severe vomiting throughout pregnancy. At four months' gestation she was told she had renal disease and was given a salt-poor diet and her intake of fluids restricted. At six months' gestation she began to have moderately severe edema of the ankles, feet, legs and hips and of the hands. Prior to delivery she was informed that her renal disease had cleared up. Throughout pregnancy the mother drank a quart of milk daily but had no additional vitamin therapy. The maternal grandmother is living at the age of 54 but has arteriosclerosis and a "bad heart."

The baby was breast fed until one week prior to death. Beginning at six weeks, he was given daily 1 teaspoon of cod liver oil fortified with percomorph liver oil (850 U. S. P. units of vitamin D per gram). The feeding of orange juice, cereal and strained foods were as usual for the age. The development was normal, and the habits were good. On close questioning, the father stated that the baby had always seemed pale in comparison with others of the same age, and the mother stated that she had changed the baby's diapers about thirty times a day.

The baby appeared to be in good health until midnight of the day before death, Dec. 31, 1946, when he became "fussy." He vomited his feeding at 7 a. m. and became cyanotic and dyspneic for a short time. A second episode of cyanosis

From the Laboratory Service and Pediatrics Section, Medical Service, Pratt General Hospital.

1. Zeek, P.: Arch. Path. **10**:417, 1930.
2. Scheidegger: Frankfurt. Ztschr. f. Path. **54**:442, 1940.
3. Brown, C. E., and Richter, I. M.: Arch. Path. **31**:449, 1941.
4. Stryker, W. A.: Am. J. Dis. Child. **71**:280, 1946.

and dyspnea followed the 11 a. m. feeding, and this continued until admission to the hospital on Jan. 1, 1947.

On admission his temperature was 98.2 F. (by rectum); the respiratory rate was 88 and the pulse rate approximately 140. He was obviously in great distress, with pallor and cyanosis of the finger tips and lips. There was suprasternal and subcostal retraction at inspiration, and decreased breath sounds over the base of the right lung posteriorly, with tubular breath sounds over the apex. The heart was of normal size, with a rate of approximately 140 per minute, and no murmurs were heard.

The blood showed erythrocytes 3,500,000, hemoglobin 11 Gm., leukocytes 14,900, with a differential count of neutrophils 55 per cent and lymphocytes 45 per cent. A roentgenogram of the chest showed a mottled nodular infiltration throughout both lung fields with somewhat greater concentration toward the hilar areas and a slight blunting of the left costophrenic angle.

Oxygen was given by nasal catheter and penicillin by intramuscular injection, and hypodermoclysis was performed. The baby's condition did not improve and he died on January 2.

The clinical impression was: acute, severe bronchopneumonia or fulminating miliary tuberculosis.

Postmortem Examination (five hours after death).—The body measured 53 cm. in crown-heel length and weighed 6.5 Kg. No sternal abnormalities were noted. The peritoneal cavity contained no excess fluid, and the abdomen generally showed no abnormalities. The right pleural cavity contained 45 cc. of clear straw-colored fluid with an occasional fleck of fibrin; the left pleural cavity, 35 cc. of similar fluid. The pericardial sac contained 7 cc. of clear straw-colored fluid. The pleural and pericardial surfaces were smooth and shiny. The transcardiac diameter was 7 cm.; the transthoracic diameter, 11 cm. The right lung weighed 81 Gm. and the left 71 Gm. Both lungs showed decreased crepitation and were mottled light and dark red. On section these areas appeared to be due to congestion. In the posterior portion of the lower lobe of each lung were several grayish red, non-crepitant areas having the appearance of atelectasis. Bronchial and vascular markings appeared somewhat more prominent than usual. The heart weighed 50 Gm. (normal, 27) and showed moderate dilatation of the right auricle and ventricle. The muscle was somewhat pale but appeared about normal in tone. The right ventricular wall measured up to 3 mm. in thickness; the left ventricular wall, up to 10 mm. The leaflets of the tricuspid valve were short, and the occlusal margins were thickened and showed many slightly rounded, moderately red nodules measuring 0.5 to 1 mm. The leaflets of the mitral valve showed similar but more marked changes. The chordae tendineae of both the ventricles appeared shortened. The appearance was that of a moderate insufficiency of the mitral and tricuspid valves. The coronary arteries were prominent and tortuous and on section showed patchy calcification with narrowing of the lumens up to 25 per cent of normal. The aorta and the pulmonary artery and the remainder of the organs showed no gross change; the thymus weighed 20 Gm., the spleen 11 Gm., the liver 245 Gm., the adrenal glands together 6 Gm. and the kidneys together 52 Gm.

Microscopic Examination.—Section of the mitral valve showed the occlusal margins of the leaflets to be somewhat irregular, with small masses of fibrous connective tissue adherent to one surface; no vegetations or ectatic blood vessels were seen. In the papillary muscle of the left ventricle there were several areas where there was necrosis of a number of muscle cells, with beginning deposition of calcium in some of them. Surrounding the necrotic areas there were a few polymorphonuclear leukocytes (fig. 1 *A*). In the wall of the left ventricle, especially in the subendocardial region, there were several various-sized linear areas of

necrosis; the muscle fibers were eosinophilic and finely granular and had lost their striations; the nuclei were pale or not seen. Some pyknotic nuclear debris was seen in these areas, and a few polymorphonuclear leukocytes were present in

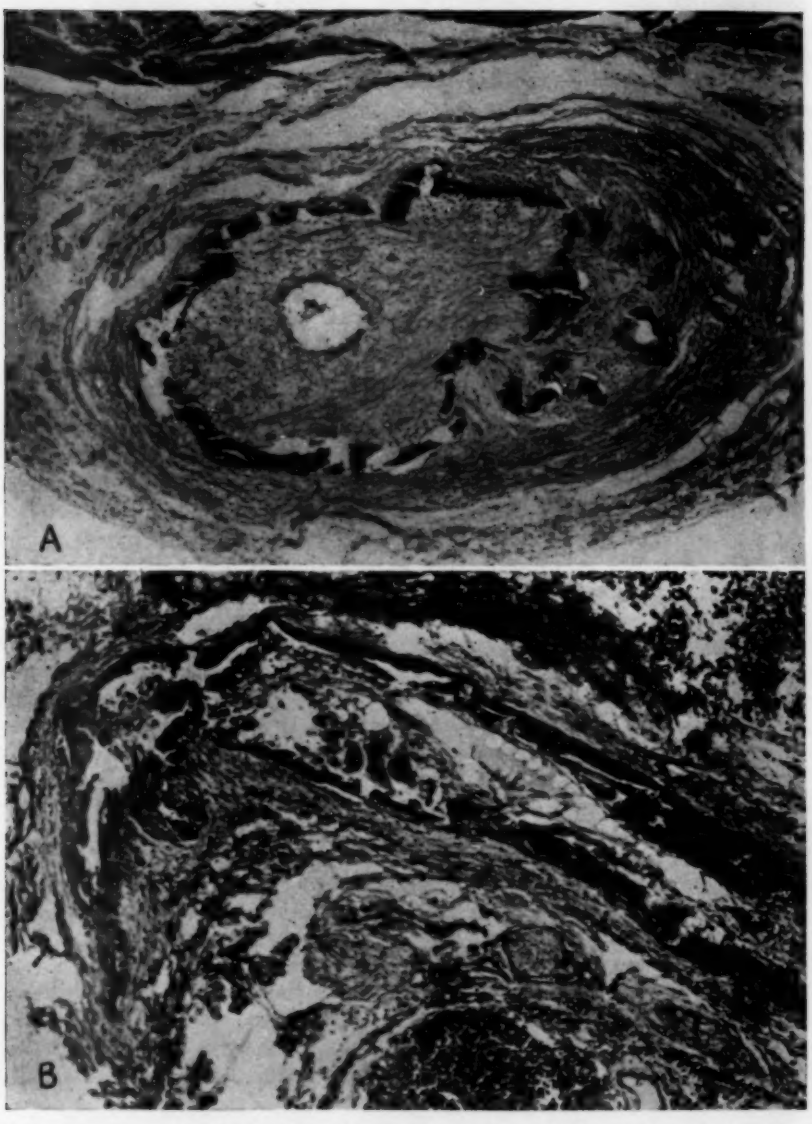

Fig. 1.—*A*, photomicrograph of the papillary muscle of the left ventricle showing a partially calcified area of necrosis; × 70. (U. S. Army Institute of Pathology Negative 187787.) *B*, photomicrograph of the coronary artery showing severe arteriosclerosis; × 70. (U. S. Army Institute of Pathology Negative 99913.)

and around them; slight hemorrhage was noted around some of the areas. The appearance was that of focal myocardial necrosis. The wall of the right ventricle showed no changes.

Sections through the various branches of the coronary arteries showed severe arteriosclerosis of the Mönckeberg medial sclerosis type (fig. 1 *B*). The lumens were greatly reduced in size—from 5 to 50 per cent of normal, approximately; one branch of the anterior descending branch of the left coronary artery was completely occluded by a fairly recent thrombus, and an offshoot of the circumflex branch was completely obliterated by connective tissue. All branches showed marked proliferation of the subendothelial loose connective tissue, which often contained a few capillaries; in many areas the cells contained various-sized vacuoles, which presumably were of lipoid nature. In most of the sections the internal elastic lamina was frayed or broken but could still be identified; deposited on it was a thick irregular layer of calcium salts which partially or completely encircled the vessel and completely replaced the smooth muscle of the media—in some areas a small portion of the media was preserved. The adventitia occasionally showed an increase in collagenous connective tissue; frequently there was spotty lymphocytic infiltration, with sometimes a few plasma cells and occasional polymorphonuclear leukocytes.

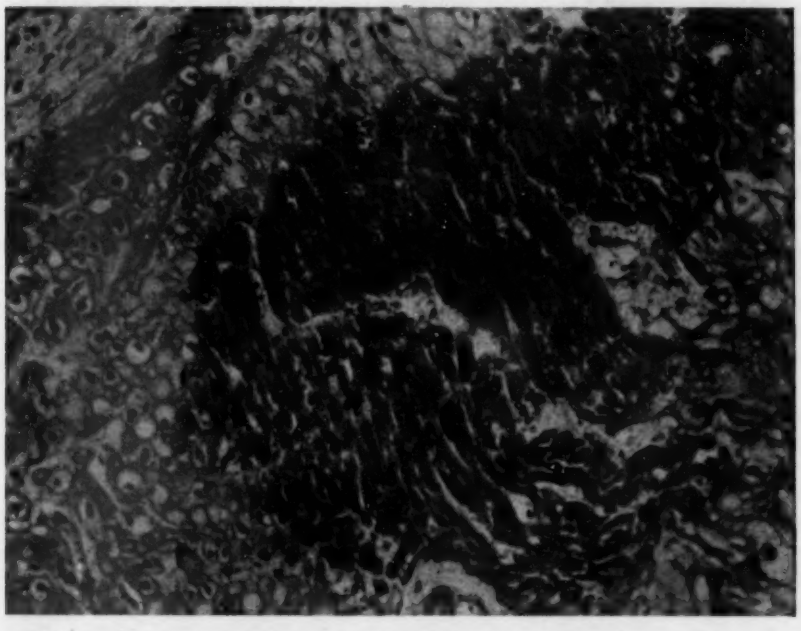

Fig. 2.—Photomicrograph of a medium-sized artery in the connective tissue adjacent to a tracheobronchial lymph node, showing giant cells of the foreign body type closely approximated to the calcified internal elastic lamina; × 160. (U. S. Army Institute of Pathology Negative 99917.)

Medium-sized arteries in the connective tissue adjacent to the thyroid gland, in the connective tissue adjacent to the pancreas, in the periadrenal fat, at the hilus of the spleen, in the mesentery and in the renal pelvis showed changes similar to those described in the coronary arteries. In the connective tissue adjacent to a tracheobronchial lymph node was seen a medium-sized artery cut in longitudinal section (fig. 2); it showed changes similar to those in the coronary arteries and, in addition, numerous giant cells of foreign body type in close approximation to the intimal side of the zone of calcification around the internal elastic lamina.

The lungs showed severe passive congestion with small patchy areas of atelectasis.

The other organs showed no significant abnormalities.

COMMENT

Brown and Richter [8] have discussed the etiologic possibilities of infantile arteriosclerosis; their case closely resembles that reported here. The history that possibly there was renal disease of the mother during gestation is too vague to be given much weight in an account of the development of the arteriosclerosis of the infant. The suggestion of polyuria conveyed by the mother's comment concerning excessive use of diapers had no corroboration in the gross and microscopic appearance of the kidneys. While the daily dose of vitamin D given the baby was above the optimum, such dosage is not unusual and probably has no bearing on the case. Unfortunately, no attempt was made to dissect out the parathyroid glands at autopsy, but the fact that they were not observed on total removal of the thyroid gland suggests that they were not grossly enlarged. Although no sections of bones were taken, a roentgenogram of the chest taken ante mortem showed no osseous changes, and the sternum, the costal cartilages, the ribs and the vertebrae did not show gross changes at autopsy.

SUMMARY

A case of severe arteriosclerosis of a 3½ month old infant is presented. As in the majority of recorded cases, no definite etiologic factor could be identified.

PRIMARY OVARIAN PREGNANCY

ESTHER H. DALE, M.D., DETROIT

CASES of genuine primary ovarian pregnancy are rare enough so that it is worth while to report such a case when it does occur.

REPORT OF A CASE

The patient was a 34 year old white woman who had been married for sixteen years and had one child, who was living and well. She had experienced one miscarriage, ten years previous to the present history. In November 1946 she failed to menstruate. In December she began to have hemorrhage and passed many blood clots, but observed no membranes in the material passed. Dilatation and curettement were done. Microscopic examination of the curetted material showed proliferative endometrium, but no chorionic villi or decidual cells were found in the sections. Following the curettage, bleeding continued sporadically for three weeks, then ceased spontaneously.

In January this patient consulted the physician again, complaining of a persistent dull aching sensation in the lower part of the abdomen. Laparotomy was done, with removal of the uterus, tubes and ovaries. The uterus was normal in size and appearance, and requires no further description. The tubes were somewhat congested but not more so than could be accounted for by the operative procedure. They were not appreciably enlarged, and the fimbriated end of each tube was patent. The tubes were distinctly separate from the ovaries. The left ovary was slightly enlarged and was partially cystic. The cysts contained a clear straw-colored fluid.

The right ovary was globular in shape and measured 5 cm. in diameter. Its surface was fairly smooth and showed no tears or ruptured areas. Its color was a dark reddish brown, like that of endometrial cysts, and indeed the general appearance of the organ suggested an endometrial cyst which has displaced and replaced the normal ovarian tissue. Routine sections were made of the wall of the cyst and of the hemorrhagic mass filling the lumen. Microscopic examination of these sections revealed the unmistakable presence of young chorionic villi. The embryo has not been recognized either grossly or microscopically, but serial sections were not made. However, it is well to know that, according to Curtis,[1] destruction of the ovum may occur within the follicle at an early period.

It is no part of my intention to review all the literature on the subject of ovarian pregnancy. But it would not be out of order to draw attention to a few of the salient and interesting facts in this connection.

According to Novak,[2] there are about 50 reported cases in which the diagnosis of ovarian pregnancy is based on acceptable evidence.

From the Department of Pathology, Wayne University College of Medicine.

1. Curtis, A. H.: Obstetrics and Gynecology, Philadelphia, W. B. Saunders Company, 1933, vol. 3, pp. 386-387.

2. Novak, E.: Gynecological and Obstetrical Pathology, Philadelphia, W. B. Saunders Company, 1940.

Spiegelberg [3] formulated certain criteria which a case must fulfil if it is to be considered one of true primary ovarian pregnancy. These are as follows:

1. The tube, including the fimbriated end, must be intact and must be distinctly separate from the ovary. Norris [4] added that the tube must show no microscopic evidence of pregnancy.

2. The gestational sac must definitely occupy the normal position of the ovary.

Fig. 1.—Photomicrograph of large chorionic villi as seen in the blood mass. × 185.

3. The gestational sac must be connected with the uterus by the utero-ovarian ligament.

4. Unquestionable ovarian tissue must be demonstrable in the walls of the sac; according to Williams,[5] it must be found at several places

3. Spiegelberg, O.: Arch. f. Gynäk. **13**:73, 1878.

4. Norris, C. C.: Surg., Gynec. & Obst. **9**:123, 1909.

5. Williams, J. W.: Obstetrics, New York, D. Appleton and Company, 1903, p. 537.

in the wall, at some distance from each other. Williams felt this requirement to be necessary because in certain cases of tubal or broad ligament pregnancy the ovary may become flattened out and to a certain extent incorporated in the sac wall.

The foregoing criteria are fulfilled perfectly in the case under discussion. The first three are gross features and cannot be demonstrated now that the specimen has been cut. If any one had suspected that the specimen represented other than an ordinary endometrial cyst, it could

Fig. 2.—Photomicrograph showing a part of the wall of the cyst. Part of a follicular cyst is seen at one edge. × 160.

have been photographed, but this was not done. The fourth criterion is easily fulfilled, since unmistakable ovarian tissue has been seen in all parts of the sac wall, with corpora albicantia, follicular cysts, primitive follicles and, of course, the typical ovarian stroma.

By some writers,[6] ovarian pregnancies have been broadly classified into two types. In one of these the ovum is surrounded by a corpus

6. Studdiford, W. E., and Lardaro, H. H.: Am. J. Surg. **33**:566, 1936.

luteum wall or is in close relationship with a corpus luteum. In the other type no such relationship can be observed. The ovarian pregnancy reported here belongs in the second of these two categories. It is quite possible and even probable that such pregnancies have their beginning in the follicle, but the development of the trophoblast has resulted in the destruction of the lutein cells and their consequent disappearance. In some cases of ovarian pregnancy the presence of a decidual reaction has been reported. But Jordan [7] expressed the opinion that the cells which have been interpreted as decidual in these cases are in reality lutein cells of the almost destroyed corpus luteum. I was unable to observe any areas which I considered evidence of a decidual reaction.

Other more elaborate classifications of ovarian pregnancies have been set up, as for example the classification proposed by Stux.[8] He listed the following four types:

1. Intrafollicular. This is the most common form. The ovum is retained in the follicle, becomes fertilized there, implants itself and develops. Why the ovum does not escape from the ruptured follicle can be merely a matter of conjection.

2. Superficial. On rupture of the follicle, the ovum reaches the surface of the ovary, is fertilized and becomes implanted there, owing to some anatomic feature of the particular ovary, such as furrows, sulci, wrinkles, thickening of the ovarian capsule, small areas of cystic degeneration or endometriosis.

3. Interstitial. In this form nidation takes place in the interstitial part of the ovary, outside the follicle. The train of events is thought to be substantially as follows: Owing to a tough ovarian capsule, an overdistended follicle bursts laterally, and the ovum escapes into the interstitial substance of the ovary. Then a surface rupture of the same follicle also occurs, through which the sperm enters the follicle and then finds its way to the ovum lying outside of the follicle wall. This seems complicated and involved but is believed to be the explanation of those cases in which the trophoblast and the corpus luteum are found next to each other in a lateral relationship, in contrast to the fourth type which is called,

4. Suprafollicular. This is actually a form of superficial nidation. A superficial blood clot at the opening of the ruptured follicle holds the ovum at its exit, where it is fertilized, becomes implanted and develops.

The duration of an ovarian pregnancy is usually longer than that of a tubal pregnancy because the ovary is a more elastic and resilient organ than the tube; it is capable of far more distention and stretching, as may be easily seen from the large size which ovarian cysts often attain.[9] In some cases ovarian pregnancy has been known to go on to full term, with a living child. In other cases the end result has

7. Jordan, H. E.: Surg., Gynec. & Obst. **54**:485, 1932.

8. Litzenberg, J. C., in Nelson Loose Leaf Living Surgery, New York, Thos. Nelson & Sons, 1941, vol. 7, p. 536.

9. (*a*) Eckerson, E. B.: Am. J. Surg. **54**:487, 1941. (*b*) Schumann, E. A.: Extra-Uterine Pregnancy, New York, D. Appleton and Company, 1921.

been a lithopedion. In a series of 38 cases in which the pregnancy lasted for seven months or longer, there were 12 in which it resulted in a living infant. In 22 cases the mother lived, and in 8 cases both mother and baby lived.[10]

The known incidence of true primary ovarian pregnancy is low. At St. Luke's Hospital in New York, 339 cases of ectopic pregnancy included one ovarian pregnancy. This is an incidence, in this series, of 0.29 per cent.[9a]

A fair number of cases have now been reported. But since there may be early rupture of the sac or early degenerative changes without rupture, it is quite likely that some cases are not recognized as such.[11]

SUMMARY

The history and the pathologic observations in a case of true ovarian pregnancy are reported.

The criteria of true ovarian pregnancy set up by Spiegelberg and amplified by Williams and Norris are recapitulated.

Classifications of ovarian pregnancies are briefly reviewed.

10. Nicholls, R. B.: Am. J. Obst. & Gynec. **42**:341, 1941.
11. Titus, E. W.: Am. J. Obst. & Gynec. **38**:516, 1939.

Laboratory Methods and Technical Notes

A METHOD OF DECALCIFYING BONE FOR HISTOLOGIC SECTION

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DALLAS, TEXAS

THE CLINICIAN, as well as the pathologist, has been hampered by the abnormally long periods of time necessary for decalcification of bone and the lack of uniformity of the sections finally produced. Reports are generally confined to the acids, organic, inorganic or a combination, used as the decalcifying agents. At times other substances, such as phlorglucinol or mercury bichloride, have been added in order to lessen destruction of the tissue. Thus, Clapp [1] reported using nitric acid with phlorglucinol; McNamara [2] used a combination of nitric and trichloroacetic acids, and De Galantha [3] used nitric acid with trinitrophenol. Kramer and Shipley [4] reported on the affinity of magnesium citrate for calcium and on its use as a decalcifying agent. Similar use of citrate solutions has been reported by Shelling and Halpersohn [5] and Evans and Krajian.[6] In a review of the commonly used methods, Jaffe [7] and McClung [8] pointed out the disadvantages of the consumption of time and the destruction of tissue frequently encountered.

Attempts to speed up the decalcification of bone by increasing the concentrations of the reagents have resulted in swelling of the fibers and destruction of the cells. To minimize these untoward reactions and at the same time speed up the reaction G. H. Wilson [9] and R. A. J. Wilson [10] suggested that a vacuum be used over various acid solutions, the reaction being thereby physically speeded up. By this method the time necessary for the decalcification of some specimens had been cut to twenty-four hours. The time consumed by the other methods enumerated varied from several days to several weeks.

The need of a method both simple and reliable by which bone could be decalcified speedily without destruction of tissue led to the following investigation.

From the Department of Pathology, Baylor University Hospital Laboratory.

1. Clapp, M. P.: Am. J. M. Tech. **1**:31, 1935.
2. McNamara, W. L., and others: J. Lab. & Clin. Med. **25**:874, 1940.
3. De Galantha, E.: J. Tech. Methods **17**:72, 1937.
4. Kramer, B., and Shipley, R.: Science **66**:485, 1927.
5. Shelling, D. H., and Halpersohn, M. F: Arch. Path. **5**:835, 1928.
6. Evans, N., and Krajian, A.: Arch. Path. **10**:477, 1930.
7. Jaffe, H. J.: Arch. Path. **8**:817, 1929.
8. McClung, C. E.: Handbook of Microscopic Techniques, New York, Paul B. Hoeber, Inc., 1937.
9. Wilson, G. H.: J. Path. & Bact. **39**:531, 1934.
10. Wilson, R. A. J.: Am. J. Clin. Path. (Tech. Supp.) **12**:79, 1942.

Bone is composed of an organic matrix in which mineral salts are deposited. Mature bone is composed 50 per cent of water and as much as 24 per cent of fat. The dried fat-free material consists 30 to 40 per cent of organic matter, the remainder being a complex salt mixture. Analytic studies of the mineral composition of bone have given results that conform to the empiric formula

$$n\ Ca(PO_4).CaX$$

where *n* varies between 2 and 3 and *X* may be carbonate, oxide, fluoride, chloride or sulfate. Roentgen ray diffraction studies show the bones to have an atomic arrangement similar to that of podolite, a mineral of the apatite series.[11]

The exact method by which calcium is deposited in bone is still under investigation. It appears, however, that a vital process of the

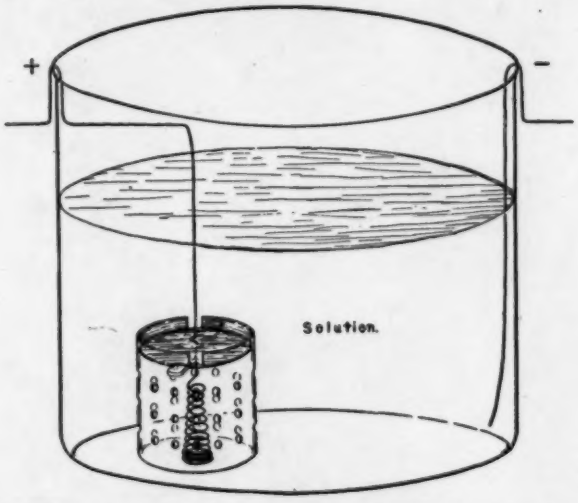

Fig. 1.—Bone container placed in the decalcifying solution. Note the arrangement of the cover and of the electrodes.

tissue is at least in part responsible. On the other hand, calcium would seem to be best removed from bone by any reaction which increases ionization of the calcium. Of the various means of speeding up this chemical reaction without cellular destruction, a process involving the following method was found to be most suitable.

DESCRIPTION OF METHOD

The devised method employs the forced migration of the positively charged calcium to a negatively charged electrode or a "deplating" of the calcium carbonate and phosphate from the bone. The apparatus used is shown in figure 1. The bone container is made of plastic material with a locking lid. Multiple holes in the container facilitate fluid exchange about the bone specimen. The platinum wire is coiled to impinge on and hold fast the bone specimen.

11. Grollman, A.: Diseases of Bone, New York, Oxford University Press, 1939.

Any source of direct current may be used. Good results have been obtained by the use of a 6 volt storage battery. A constant source is produced by the use of a rectifier attached to the regulation 110 AC circuit. With the use of a rheostat the amperage may be controlled to produce desirable results.

The vessel containing the electrolytic bath should be of glass and large enough to hold the necessary electrodes without crowding. The bath while in service

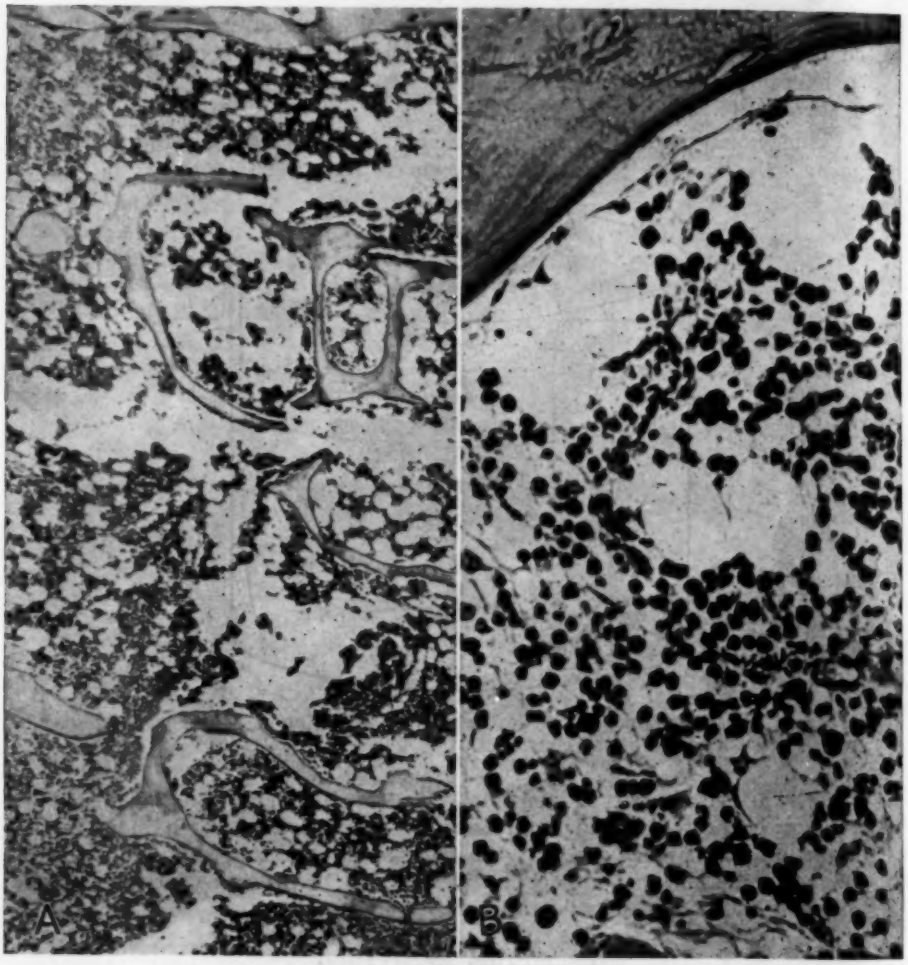

Fig. 2.—*A*, section of sternum 1 cm. in thickness decalcified in three hours with preservation of marrow. Hematoxylin and eosin stain; × 42.

B, detail of marrow cells in section shown in *A*. × 250.

should be controlled at temperatures between 30 and 45 C. Higher temperatures not only increase the speed of reaction but cause disintegration of the tissue. A large vessel lessens the chance of overheating and may be used continuously without control over the period of decalcification.

The electrodes should be made of platinum, hard carbon or tungsten. Experiments with electrodes made of iron, copper, brass and "castalloy" had shown that these metals were readily dissolved, the electrolysis having a much greater affinity for the anode than for the bone.

Any suitable reagent that can be placed into an aqueous solution which will dissociate the calcium from the carbonate and phosphate ions may be used.

Solutions of various substances that would react with the inorganic constituents of the bone have been tried. Although comparatively few of these substances were used before desirable results were obtained, the following ones suggested themselves:

(*a*) Oxidizing substances, such as chromates and nitrates. These rapidly destroyed the organic matter.

(*b*) Organic acids and their corresponding salts. These, because of their low degree of ionization and conduction, produced decalcification at a slow rate.

(*c*) Carbon dioxide in high concentrations. This retarded the rate of decalcification.

(*d*) Inorganic salts. Although producing good conduction and having some affinity for calcium they removed this element at a slow rate.

(*e*) Nonoxidizing and highly ionizable acids that produce water-soluble compounds of calcium. These acids produce good decalcification.

In an attempt to "fix" and decalcify simultaneously, a mixture of hydrochloric acid and formaldehyde was used. Very poor removal of calcium and some disintegration of tissue took place. The assumption that formic acid produced from the formaldehyde was responsible for the untoward reaction led to the use of various concentrations of hydrochloric and formic acids. The results were surprising. The best decalcification over the shortest period of time without disturbance of tissue had been obtained with a 10 per cent solution of formic acid and an 8 per cent solution of hydrochloric acid. Repeated use of 10 per cent formic acid and 8 per cent hydrochloric acid continued to produce desirable results (fig. 2). It is assumed that the formic acid softens the tissue and allows the acid reaction to take place more rapidly. Higher concentrations of formic acid and/or excess contact with the specimen produces disintegration of the tissue.

Proper decalcification of all bone samples treated with these two acids in the electrolytic bath is generally accomplished in two to six hours. Care must be taken not to allow the bone specimen to remain in the decalcification bath longer than necessary. Overtreatment, especially in the presence of heat, will bring about destruction of tissue.

Following decalcification, the bone is cleared in aniline and immediately placed in paraffin. Prior to staining, the slides are dipped once into a 1 per cent solution of lithium carbonate for adequate neutralization of the acid decalcifying agent.

SUMMARY

A very effective means for removing calcium compounds from bone with minimal injury of tissue has been shown to result from the use of an electrolytic bath with 8 per cent hydrochloric acid and 10 per cent formic acid as the electrolytic medium.

A RAPID PROCEDURE FOR THE EMBEDDING OF EYES

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THE ROUTINE preparation of eyes for microscopic study is a notoriously slow process. This is due to the fact that rapid methods of dehydration and embedding cause shrinkage and consequent distortion. The value of a rapid method for diagnostic purposes is obvious, especially in cases of suspected malignant tumors. Furthermore, experimental work might be retarded for weeks or months by the waiting for sections. The problem, therefore, was to find a water-soluble embedding medium whereby dehydration and consequently shrinkage would be avoided and the time of preparing sections would be shortened considerably.

Among the many synthetic products of modern chemical industry, "carbowax" compounds (solid polyethylene glycols, molecular weight above 1,000) appeared to be particularly suitable as embedding mediums because of their chemical inertness, noncorrosive nature, good thermal stability, high aqueous solubility, high molecular weight and low hygroscopicity. They do not support the growth of molds and do not deteriorate. They are manufactured in various stages of polymerization by the Carbide and Carbon Chemicals Corporation.[1] With increasing molecular weight the aqueous solubility and the hygroscopicity decrease and the melting point rises. One of the series, "carbowax 4000," has been used for preparing sections of muscle for electron microscopy[2] but cannot be used in the preparation of eyes because of the difficulties encountered in mounting the sections on slides. Since "carbowax" compounds are highly soluble in water and in aromatic hydrocarbons and, to a somewhat limited extent, in aliphatic hydrocarbons, there is left only a small choice of liquids to float the sections on. Benzine and ethers do not dissolve the compounds, but the sections become extremely brittle. Mounting sections from liquid petrolatum, another nonsolvent, proved impossible.

The difficulty was overcome by first infiltrating the specimens with a "carbowax" compound and then with another less water-soluble wax. Among several "carbowax" esters, one nearly insoluble in water, "carbowax distearate," does not infiltrate tissue directly at all, but because it is miscible with "carbowax" compounds it infiltrates specimens previously infiltrated with a "carbowax" compound. (A related substance, diethylene glycol distearate, has been used for general tissue work in an embedding mixture after preceding dehydration.[3])

The method arrived at after a large number of grades of "carbowax" compounds and "carbowax" esters (supplied by the courtesy of the Carbide and Carbon Chemicals Corporation and the Mellon Institute)

From the Laboratories of the Mount Sinai Hospital.

1. McClelland, C. P., and Bateman, R. L.: Chem. & Engin. News **23**:247, 1945.

2. Richards, A. G.; Anderson, T. F., and Hance, R. T.: Proc. Soc. Exper. Biol. & Med. **51**:148, 1942.

3. Steedman, H. F.: Nature, London **156**:121, 1945.

had been tested on more than 100 specimens—human eyes and dogs' and monkeys' eyes—is as follows:

"Carbowax 1000" and "carbowax 1540 distearate" (the numbers refer to the molecular weight) are placed in the incubator at a temperature of 56 C. and allowed to melt. To 100 cc. of each is added 0.1 Gm. of "duponol C," a wetting agent derived from technical lauryl alcohol of the series of alcohol sulfates, manufactured by E. I. du Pont de Nemours, which will dissolve completely overnight. (The wetting agent shortens the time of infiltration by half and at the same time acts as an emulsifying agent, preventing excessive crystallization of the wax on solidification. All "carbowax" compounds used in this method and referred to from now on have this wetting agent added at 0.1 per cent by volume.)

The eye, fixed in Bouin's solution for two or three days, is cut in half. The half eye is placed in a large dish with distilled water for one-half hour, the distilled water being changed every ten minutes. The specimen is now transferred to a mixture of "carbowax 1000" and distilled water, equal parts, for one-half hour at a temperature of 37 C. It is then placed into four successive changes of "carbowax 1000," remaining two hours in each, all at 37 C. The carrying over of excess wax from one solution to the next is avoided by carefully removing it with filter paper. In the last change the specimen remains overnight.

Then it is placed into a mixture of "carbowax 1000" and "carbowax 1540 distearate," equal parts, for one-half hour, and then into four successive changes of "carbowax 1540 distearate," two hours each, except for the last in which the specimen remains overnight. Since the solidification point of "carbowax 1540 distearate" is higher than that of "carbowax 1000," the temperature of the incubator has to be raised to 45 C. The specimen, cut surface up, is embedded the next morning in fresh "carbowax 1540 distearate" in a paper boat such as that used for paraffin embedding. It is hardened slowly (to avoid excessive crystallization of the wax), the embedding boat being placed in the incubator at 37 C. until evening and on top of the incubator overnight.

The block is then mounted on a metal carrier block in the same manner as the paraffin block. Sections are cut with a rotary microtome. The edge of the knife should be parallel to the cornea-nerve axis of the eye. Sections can be cut as thin as 7 microns. These, however, are difficult to mount; the preferable thickness is 10 to 15 microns. The sections come off in ribbons, which are placed on paper. The ribbons are cut and the sections floated singly on a saturated aqueous solution of sucrose till they straighten out,[4] and are immediately mounted on slides. (In pure water the wax would slowly dissolve and mounting of the sections be difficult.) Excess sugar solution is drained off from the slides with small pieces of filter paper, which are left on the slides while these are drying. The slides are placed in the incubator at 37 C. for at least two hours in order to make the sections stick to the slides.

Now they are placed in dehydrated alcohol, then in alcohol and ether, equal parts, then in a 3 per cent solution of celloidin (a concentrated preparation of

4. The exact timing according to the thickness of the sections is vital in order to obtain good sections without folds. The sections should be removed just before all the folds have straightened out, as this process will continue in the incubator until the sugar solution dries. Even thick sections should not remain in the sugar solution more than two minutes, and it is preferable to add a few drops of water to the Petri dish which contains the sugar solution.

pyroxylin) in alcohol and ether, each for two minutes. Thereafter they are put in a vertical position to allow the celloidin to dry on them while the excess celloidin drains off. After five minutes they are transferred to a saturated solution of lithium carbonate in 70 per cent alcohol, in which the celloidin will harden. As soon as the sections have lost their yellow color, the celloidin cover is removed from the backs of the slides, and the slides are placed in distilled water. The celloidin comes off the slides as the sugar dissolves, and the sections stick firmly to the celloidin membrane. The celloidin is trimmed around the sections, which can immediately be stained exactly as any celloidin section is, or can be left in distilled water overnight and stained the next day.

Routine stains, such as hematoxylin-eosin, Masson's trichrome, Weigert's elastic stain, the Van Gieson stain and the Loyez or Spielmeyer myelin stain, can be applied. The sections are cleared in carbolxylene or in xylene and mounted on the celloidin membrane in "clarite" or synthetic resin.

Fig. 1.—Human optic nerve head; hematoxylin-eosin; × 55.

The first two changes of "carbowax 1000" should be discarded after use; the last two can be used as the first two for the next specimen. Of the "carbowax 1540 distearate," only the first change has to be discarded; the others may be used as successively lower changes. All bottles containing "carbowax" compounds should be stoppered, as these compounds are slightly hygroscopic.

A specimen can be kept in a 4 per cent solution of formaldehyde after having been fixed in Bouin's solution, when embedding right after fixation is not desired. Good results have also been obtained by putting the specimens after fixation into 50 per cent alcohol for two days and then into 70 per cent alcohol up to the time of embedding.

The sugar solution can be filtered and used again.

Sections should not be kept on slides longer than twenty-four hours, because the crust of sugar forming on the slides will eventually disrupt the sections. They

should be removed from the slides by way of passing them through celloidin as already described and can then be kept in 70 per cent alcohol for storage. Blocks should be stored in a dry place after covering the cut surface with a thin layer of molten "carbowax 1540 distearate."

The described method does not produce any shrinkage of the specimens, there is no fungous growth and the tissue is well preserved (fig. 1). Regarding the lens, the same difficulty is encountered as in using the paraffin method, namely, brittleness and poor infiltration.

In a hematoxylin-eosin stain the cells of the layers of the retina, especially the ganglion cells, stain distinctly (fig. 2). In contrast to

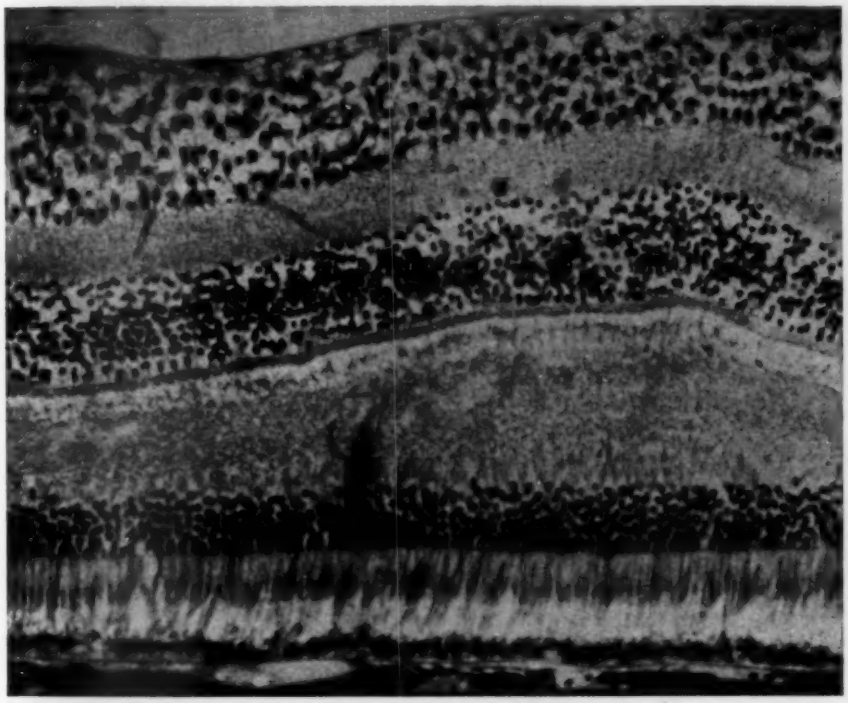

Fig. 2.—Retina of monkey; hematoxylin-eosin; × 200.

ordinary paraffin or celloidin sections, myelinated nerve fibers will stand out clearly in a hematoxylin-eosin stain. As there is no contact of the specimen with myelin solvents like xylene or chloroform, and the contact with alcohol-ether after mounting is exceedingly short, the myelin is not dissolved, and no special myelin stain is necessary. Special myelin stains, however, turn out well too, whether the Loyez or the Spielmeyer method is used.

The main advantage of the new method lies in the possibility of obtaining sections of whole eyes as thin as paraffin sections within four days after fixation, while the routine procedures require three and a half weeks in paraffin and five months in celloidin as used in this laboratory. Furthermore, by eliminating contact with alcohol and other

organic solvents, it avoids shrinkage and possible detrimental effects in some staining methods.

The method might be adapted for use in other fields of pathology. It might be applied to nerve tissue because of good myelin stains and especially to spongy tumors which fall apart when cut on the freezing microtome and cannot be stained well with myelin stains after paraffin embedding.

SUMMARY

Eyes can be embedded rapidly, with minimal shrinkage and distortion, by means of water-soluble "carbowax" (polyethylene glycol) and "carbowax" ester, used in succession. The entire process of embedding, sectioning and staining can be accomplished in four days.

The late Dr. Robert K. Lambert gave encouragement and guidance in the conduct of this work.

Books Received

GYNECOLOGY, WITH A SECTION ON FEMALE UROLOGY. By Lawrence R. Wharton, M.D., assistant professor of gynecology, Johns Hopkins University School of Medicine; assistant attending gynecologist, Johns Hopkins Hospital; consultant in gynecology, Union Memorial Hospital, Hospital for the Women of Maryland, Sinai Hospital and Church Home and Infirmary. Second edition. Pp. 1,027, with 480 illustrations. Price $10. Philadelphia and London: W. B. Saunders Company, 1947.

The first edition was issued in 1943. The section on embryology and that on congenital malformations have been rewritten, and changes and additions have been made, especially in the discusions of physiologic and chemotherapeutic matters. Recent advances in operative technic are described and illustrated. The part on urology has been enlarged. The text is well organized and clearly written. The descriptions of the morphologic characteristics of the organs in question and of their diseases are accurate and will be of great interest to the pathologist. The illustrations, many of them borrowed from classic sources, are uniformly excellent. The last chapter of the book deals with irradiation as used in the practice of gynecology and female urology, but the technical details of radiotherapeutic methods are not discussed. Should not the clinician be as interested in the details of those methods as in the details of other methods of treatment? The book maintains fully the high standards of the Hopkins school of gynecology.

SURGICAL PATHOLOGY. By William Boyd, M.D., M.R.C.P., F.R.C.P., professor of pathology, University of Toronto, Canada. Sixth edition. Pp. 858, with 530 illustrations, including 22 colored figures. Price $10. Philadelphia and London: W. B. Saunders Company, 1947.

Since 1925 a new edition of this book has been published every four or five years, each with changes and additions to keep abreast with advances in pathology. In the present edition one important topic is the pathologic anatomy and physiology of the congenital heart diseases, in the treatment of which surgery is making notable progress. The results of the continued efforts to deal adequately with all topics in pathology of special surgical interest are evident in all parts of the book. Of topics still worthy of consideration may be mentioned the pathology of the nasal sinuses, transfusion, and the surgical relations of blood grouping. The section on biopsy as employed in the diagnosis of cancer should be revised and brought up to date.

RADICAL SURGERY IN ADVANCED ABDOMINAL CANCER. By Alexander Brunschwig, M.D., professor of surgery, University of Chicago. Pp. 324, with 118 illustrations. Price $7.50. Chicago: University of Chicago Press, 1947.

This book deals with the author's methods of radical surgery and his results in 100 cases of advanced abdominal cancer. The details—clinical, pathologic, surgical—of this remarkable series are clearly described and well illustrated. Special emphasis is placed on the value of newer methods of supportive treatment. The book marks a great advance in its field.

THE PATHOLOGY OF TRAUMATIC INJURY: A GENERAL REVIEW. By James V. Wilson, M.D., M.R.C.P., Major R.A.M.C. (T), pathologist to Harrogate and District General Hospital and to the Royal Bath Hospital, Harrogate. Foreword by Philip H. Mitchiner, M.D., M.S., F.R.C.S., surgeon to St. Thomas Hospital. Pp. 192, with 61 illustrations. Price $6. Baltimore: Williams & Wilkins Company, 1946.

This monograph reviews the literature on the pathology of trauma to the human body in the light of the author's personal work and experiences in war. The first part takes up traumatic shock, the pathologic aspects of burns, crush injury—traumatic anuria, fat embolism, blast injury, the pathologic aspects of wounds and wound infection. The second part has chapters on injuries of the chest, the blood vessels, the abdomen, the nervous system, the bones and the joints. The illustrations, mostly borrowed from other writers, are well chosen and instructive. The book is a good review of the pathology of trauma and will be of valuable service to clinicians, pathologists and students.

DISEASES OF THE CHEST: WITH EMPHASIS ON X-RAY DIAGNOSIS. By Eli H. Rubin, M.D., F.A.C.P., F.C.C.P., attending physician, Division of Pulmonary Diseases, Montefiore Hospital and Country Sanatorium, New York; visiting physician in tuberculosis and physician-in-charge, Chest Clinic, Morrisania City Hospital, New York. The Principles of Surgical Treatment. By Morris Rubin, M.D., assistant visiting surgeon, Triboro Hospital and Morrisania City Hospital, New York. Pp. 685, with 355 illustrations (24 in color). Price $12. Philadelphia and London: W. B. Saunders Company, 1947.

The first section takes up anatomy and physiology as applied to diseases of the chest, and roentgenology. The second section discusses acute and chronic forms of pneumonia, with a chapter on sulfonamide compounds and antibiotics. The third section is devoted to pulmonary tuberculosis. The chronic diseases in which the bronchi are primarily concerned are the subjects of section 4, and section 5 deals with diseases of the mediastinum, the pleura and the diaphragm. Section 6 is devoted to the surgical treatment of the diseases considered in the book, which is well illustrated with roentgenograms.

MEMORANDA ON MEDICAL DISEASES IN TROPICAL AND SUB-TROPICAL AREAS. Eighth edition. Pp. 396, illustrated. Price 7 s. 6 d. London: His Majesty's Stationery Office, 1946.

This book, sponsored by the British War Office, has been carefully revised and brought into line with the advances of knowledge during the 1939-1945 war. In a form convenient for medical officers it contains some 60 or more well written and concise memoranda on the essentials of the more important tropical diseases, with instructive illustrations in the text and on plates.

COLOR ATLAS OF HEMATOLOGY: WITH BRIEF CLINICAL DESCRIPTIONS OF VARIOUS DISEASES. By Roy R. Kracke, M.D., dean and professor of clinical medicine, Medical College of Alabama, Birmingham, Ala. For Medical Students, Laboratory Technicians and General Practitioners of Medicine, with Clinical and Hematologic Descriptions of Blood Diseases, Including a Section on Terminology, a Section on Technic and a Summary of Blood Findings in Various Diseases. Pp. 204, illustrated with 32 plates in full color and 3 plates in black and white. Price $5. Philadelphia, London and Montreal: J. B. Lippincott Company, 1947.